

THE TOXICITY OF URANIUM AND PLUTONIUM
TO THE DEVELOPING EMBRYOS OF FISH

A DISSERTATION

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Graduate Studies

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SUMMARY

The radiological and chemical toxicity of plutonium and uranium to the developing embryos of fish was investigated using eggs from carp, *Cyprinus carpio*, and fathead minnows, *Pimephales promelas*. Freshly fertilized eggs were developed in solutions containing high specific activity ^{238}Pu or ^{232}U or low specific activity ^{244}Pu , ^{235}U , or ^{238}U . Quantitative tests to determine the penetration of these elements through the chorion indicated that plutonium accumulated in the contents of carp eggs reaching a maximum concentration factor of approximately 3.0 at hatching. Autoradiographs of 16 μ egg sections showed that plutonium was uniformly distributed in the egg volume. Uranium localized in the yolk material, and the concentration factor in the yolk sac remained constant during development at approximately 3.3. Doses from ^{238}Pu which affected hatchability of the eggs were estimated to be 1.6×10^4 rads and 9.7×10^3 rads for *C. carpio* and *P. promelas*, respectively; doses from ^{232}U were 1.3×10^4 rads for *C. carpio* and 2.7×10^3 rads for *P. promelas*. A greater number of abnormal larvae than in control groups was produced by ^{238}Pu doses of 4.3×10^3 rads to carp and 5.7×10^2 rads to fathead minnows; 3.2×10^3 rads and 2.7×10^2 rads were estimated from ^{232}U . Eggs that were incubated in 20 ppm ^{244}Pu did not hatch. This mortality may have been the result of chemical toxicity of plutonium. Concentrations of 60 ppm of ^{235}U and ^{238}U did not affect egg hatching.

Based on these data, concentrations in fish eggs were calculated for representative concentrations of uranium and plutonium in natural waters and the corresponding dose levels are below those levels at which observable effects begin to occur.

CHAPTER I

INTRODUCTION

Definition of the Problem

Very little experimental data are available in the literature concerning the effects that plutonium and uranium have on aquatic biota. Metabolic and environmental transport properties of these elements have been of great interest to scientists for a number of years. Research to assess the impact of plutonium and uranium in terrestrial and aquatic ecosystems must proceed concomitant with the development of the nuclear industry.

This research focuses on one type of aquatic biota, the fish egg. Specifically, the problem is (a) to develop a means of determining the relative toxicity of plutonium and uranium in aquatic ecosystems and to evaluate fish eggs as such an indicator of toxicity; and (b) to use this information to compare the radiotoxicity of various plutonium and uranium nuclides in aquatic systems.

Plutonium in the Aquatic Environment

The behavior and effects of plutonium in the environment have been of great interest and intense study since plutonium was first isolated as an element in 1941. Several recent publications and symposia have dealt specifically with the metabolic, environmental, and chemical properties of plutonium.^{1,2,3,4,5,6} These efforts are rapidly making

plutonium one of the most extensively studied and well documented of the radioelements. However, much more information is needed before science can determine conclusively the total effect that plutonium has on man and other biota.

Plutonium does not occur naturally; the plutonium that is currently on the surface of the earth has come from four major sources. Nuclear weapons testing contributed approximately 320 kCi of ^{239}Pu and 10 kCi of ^{238}Pu .⁷ The loss of a SNAP 9A satellite power source distributed 17 kCi of ^{238}Pu .⁸ Nuclear weapons accidents left some plutonium at various sites. Finally, the nuclear fuel cycle annually releases small quantities of plutonium to the environment. Hanson⁹ divided these sources into two categories, controlled and uncontrolled; the terminology refers to man's ability to contain plutonium. With the expansion of nuclear power and the impending development of the breeder reactor, plutonium will continue to be produced in large quantities and may potentially enter the biosphere.

Plutonium enters the aquatic ecosystem through the biogeochemical cycle for both controlled and uncontrolled releases. An aquatic ecosystem may be divided into the basic components that are illustrated in Figure 1. The initial entry of plutonium into this ecosystem may occur through controlled or uncontrolled releases to the atmosphere or water, or through uncontrolled releases to soil or bedrock from storage sites. Controlled releases to the atmosphere are typically very small; however, the controlled disposal of large quantities of plutonium in water as radioactive waste, particularly into the ocean, is not uncommon. Some areas of the North Sea contain plutonium at measured concentrations

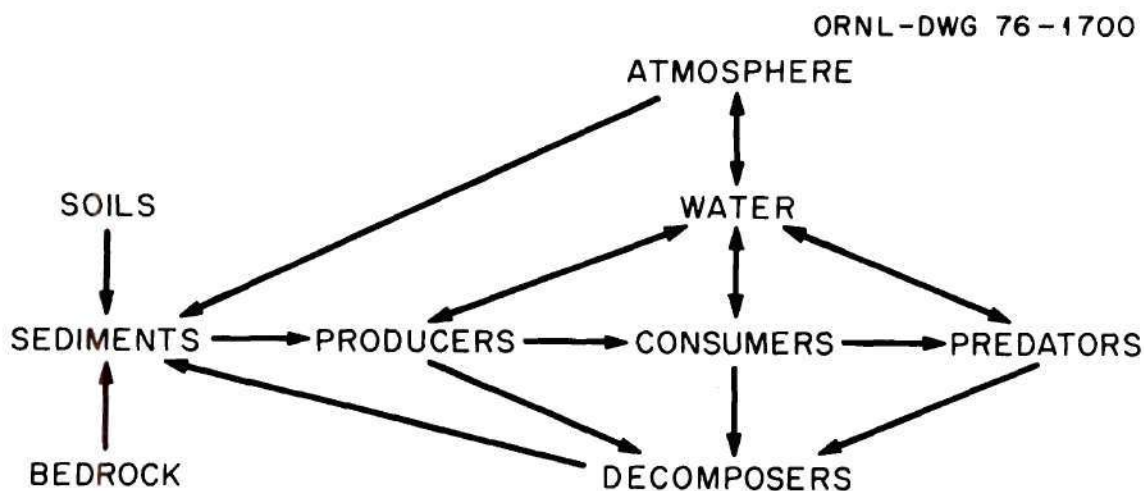


Figure 1. Components of an Aquatic Ecosystem

of 5-15 times higher than in the North Atlantic.¹⁰ The disposal of plutonium off the English Coast at a rate of 1.2×10^3 curies per year has been reported by Heatherington et al.¹¹ Also, recent measurements have shown that plutonium may migrate from disposal areas through hydrological formations.¹²

The literature contains very little information on the effects of plutonium on aquatic biota. Also, the chemical behavior of plutonium in aquatic systems is still not well understood. The ecological aspects of plutonium in aquatic environments are reviewed in a paper by Noshkin.^{13,14} In his report Noshkin points out that essentially all published work has been concerned with fallout levels of plutonium in

the marine environment; he suggests that these data should be used conservatively to assess the distribution and behavior of plutonium that enters the marine environment from sources other than fallout. Noshkin also shows that fallout ^{239}Pu contributes more than fallout ^{90}Sr or ^{137}Cs to the artificial radiation exposure of many marine species.

In order to further investigate the effects of plutonium in the environment, a study of the fish embryo, a highly radiosensitive representative of the aquatic biota, has been selected for this research.

The Significance of Research on High Specific Activity Uranium

As discussed in the previous section, the need for further research on plutonium has been well established. Since the discovery of uranium in 1789 and particularly in the last half century, extensive knowledge about its biological, environmental and chemical properties has been accumulated. Also, with the current interest in nuclear energy, uranium has become one of the best understood and documented elements in the periodic table. However, less is known about its toxicity than other aspects and the reason for additional research on uranium, particularly high specific activity uranium, will be discussed in depth in this section.

This author has evaluated the radiological impact of uranium used in the thorium fuel cycle. The results of these studies have been reported in the literature^{15,16,17,18} and will be reviewed. The anticipated use of recycle ^{233}U as a fissile material to supplement ^{235}U in the nuclear fuel cycle has been demonstrated to be both economical and

feasible. Also, the breeding potential of ^{233}U fuels require that this source of energy be utilized to its fullest extent.^{19,20,21}

Figure 2 illustrates the High Temperature Gas-Cooled Reactor (HTGR) fuel cycle which uses thorium as the fertile material. Neutron capture in ^{232}Th and beta emission of ^{233}Th and ^{233}Pa lead to the production of ^{233}U which is separated during reprocessing, then refabricated and recycled back to the reactor to compose approximately 37% of the fuel elements.

Because ^{238}U and ^{235}U have very long radioactive half lives and consequently very low specific activities, the maximum permissible concentration (MPC) values established by the International Commission on Radiological Protection (ICRP) are determined by chemical toxicity rather than by the effects of radiation. Corresponding MPC values for the shorter lived uranium isotopes (^{230}U , ^{232}U , ^{233}U , ^{234}U , ^{236}U , and ^{240}U) are determined by radiobiological effects.^{2,22} The critical organ for longer lived isotopes is the kidney while the bone is the critical organ for shorter lived isotopes.

Uranium-233 fuel may contain up to approximately 1200 ppm ^{232}U which significantly increases the radiotoxicity of a unit mass of ^{233}U fuel. The radiological importance of ^{232}U in ^{233}U fuel was illustrated by this author in a comparison of the potential radiological impact resulting from the release of equivalent masses of ^{233}U HTGR fuel and Liquid Metal Fast Breeder Reactor (LMFBR) plutonium fuel into the environment.^{15,16} The equilibrium recycle atom fraction and activity assumed

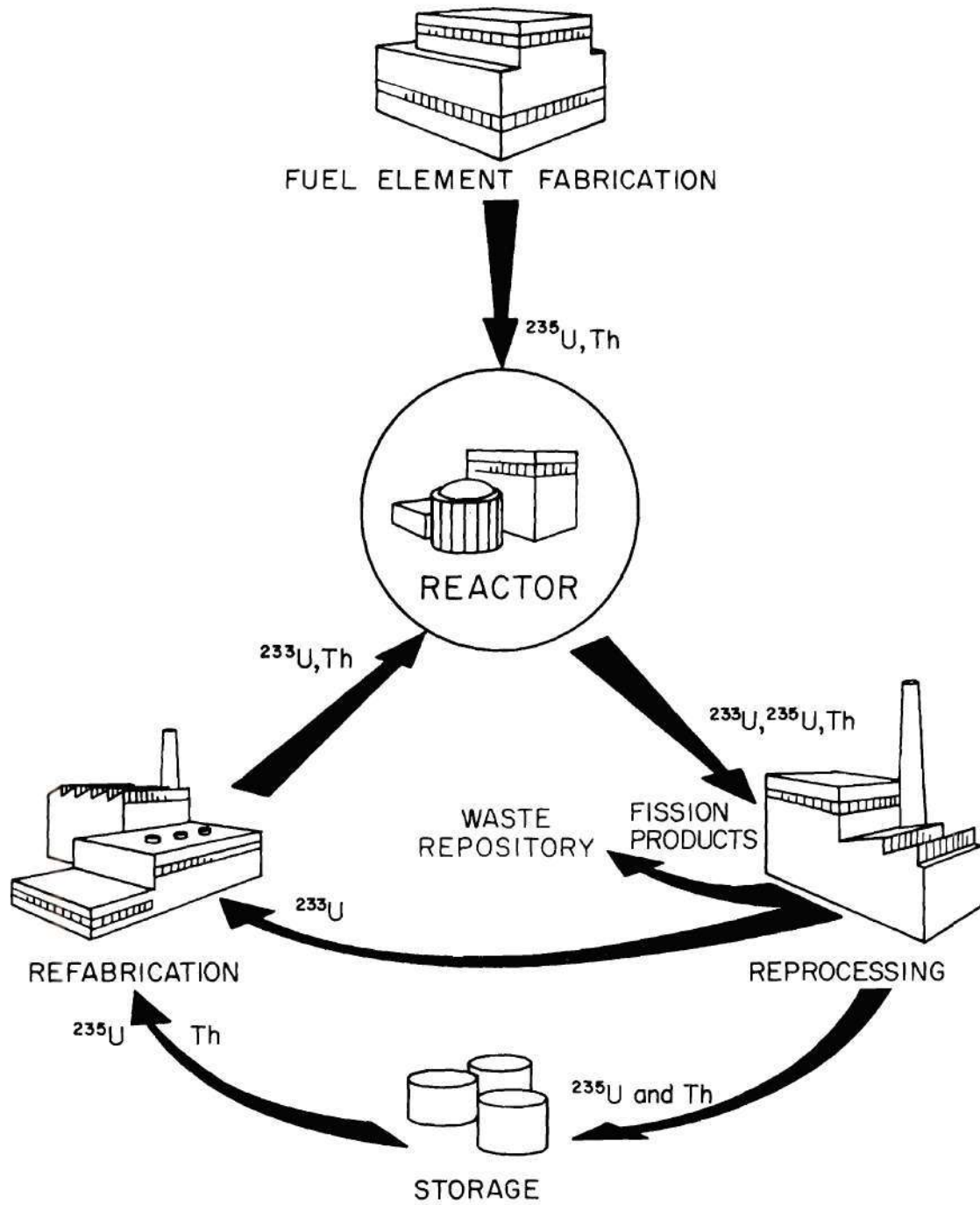


Figure 2. HTGR Fuel Cycle

to be present in one gram of recycled HTGR fuel are listed in Table 1a.²³ Table 1b shows the isotopic composition and activity of one gram of LMFBR plutonium fuel.²⁴

The study was made by comparing the radiological impact in terms of dose to man and other biota resulting from equivalent masses of HTGR fuel and LMFBR fuel which are assumed to be released to the environment. The INREM²⁵, BIORAD²⁶, AIRDOS²⁷, and TERMOD²⁸ computer codes developed by the Analysis and Assessments Section, Environmental Sciences Division, of the Oak Ridge National Laboratory were used for this comparison. The results of that study are summarized in the following paragraphs.

Table 2 shows estimated 50-year dose commitments for total body, bone, liver and kidneys for ingestion of one microgram of fuel obtained by the INREM code. Plutonium fuel contributes the maximum dose to bone, 320 mrem/ μ g. The uranium/plutonium dose ratio is 0.31; that is, uranium gives 31% as much dose as the plutonium. Total-body doses for the two fuel types are very similar ($U/Pu = 0.95$). Uranium-232 in HTGR fuel contributes 90% or more of the dose to the total body, bone, and kidneys. The dose from plutonium fuel is more evenly divided between ^{238}Pu , ^{239}Pu , ^{240}Pu , and ^{241}Pu .

The ingestion pathway results from consumption of food products contaminated with uranium or plutonium originally deposited on the terrestrial surface. It is recognized, however, that this pathway of exposure would not be the critical exposure pathway when releases are to the atmosphere. Ingestion may be the dominant exposure following shutdown of a nuclear facility or in analysis of long-term impacts.

Table 1a. Assumed Activity of Uranium
Isotopes in 1.0 Gram of ^{233}U Fuel²³

Isotope	Beginning Recycle Atom Fraction	Equilibrium Recycle Atom Fraction	Beginning Recycle Act. (Ci)	Equilibrium Recycle Act. (Ci)
Uranium Fuel				
^{232}U	<0.00100	0.00100	$<2.14 \times 10^{-2}$	2.14×10^{-2}
^{233}U	0.919	0.612	8.74×10^{-3}	5.82×10^{-3}
^{234}U	0.0735	0.243	4.55×10^{-4}	1.51×10^{-3}
^{235}U	0.00568	0.0802	1.22×10^{-8}	1.72×10^{-7}
^{236}U	0.000245	0.0630	1.56×10^{-8}	3.99×10^{-6}

Table 1b. Assumed Activity of
Plutonium Isotopes in 1.0 Gram
of LMFBR Fuel²⁴

Isotope	Atom Fraction	Activity (Ci)
Plutonium Fuel		
^{236}Pu	4.2×10^{-8}	2.24×10^{-5}
^{238}Pu	0.012	2.09×10^{-1}
^{239}Pu	0.647	3.97×10^{-2}
^{240}Pu	0.245	5.55×10^{-2}
^{241}Pu	0.058	6.52×10^0
^{242}Pu	0.038	1.48×10^{-4}
^{244}Pu	1.8×10^{-7}	3.48×10^{-12}

Table 2. Comparison of Estimated Doses from Ingestion of 1.0 μg of Recycled ^{233}U Fuel or LMFBP Plutonium Fuel Using INREM

	50-Year Dose Commitment (mrem)			
	Total Body	Bone	Liver	Kidneys
Recycled ^{233}U Fuel	7.0×10^0	9.9×10^1	7.0×10^0	1.1×10^1
LMFBP Pu Fuel	7.4×10^0	3.2×10^2	3.5×10^1	3.3×10^1
U/Pu Dose Ratio	0.95	0.31	0.20	0.34

To estimate environmental dose levels, the BIORAD computer code was used which calculates internal and external doses to aquatic plants, invertebrates, fish, and terrestrial animals dependent on aquatic food supplies. In this code, the uranium and plutonium are assumed to be in a soluble chemical form. A comparison of uranium and plutonium fuel was made by assuming that radionuclide concentrations in the water were proportional to concentrations in the fuel. A fuel concentration in water of 10^{-12} $\mu\text{g/ml}$ was assumed. Uranium-232 daughters other than ^{228}Th were not included in the total dose calculation since these isotopes could not be handled in the BIORAD code when the comparison was made.

The estimated internal doses to biota from uranium and plutonium fuel are listed in Table 3. The uranium dose was found to be 25% to 65% of the plutonium dose even though plutonium fuel was assumed to be present in much higher concentrations. The most significant contributor to the uranium dose is ^{232}U , which gives from 50% to 90% of the total.

Table 3. Comparison of Estimated Internal Dose Rate to
Biota from ^{233}U HTGR Fuel and LMFBR Plutonium Fuel

	Concentration in Water ($\mu\text{Ci/ml}$)	Dose Rate (mrad/year)			
		Aquatic Plants	Invertebrates	Fish	Muskrats
^{233}U HTGR Fuel	3.0×10^{-14}	1.2×10^{-1}	1.5×10^{-2}	1.4×10^{-3}	2.4×10^{-4}
Pu LMFBR Fuel	6.8×10^{-12}	2.1×10^{-1}	5.9×10^{-2}	2.1×10^{-3}	8.9×10^{-4}
U/Pu Dose Rate Ratio		0.59	0.25	0.65	0.27

Table 3 also lists the U fuel/Pu fuel dose ratios and clearly shows that these two fuels are similar to each other when potential doses to biota other than man are compared.

Table 4 shows the results of the comparison of estimated 50-year dose commitments to man from ^{233}U HTGR fuel and LMFBR plutonium fuel which are assumed released to the atmosphere at a release rate of 10^{-12} g/sec. Dose commitments are listed for submersion in air, ground exposure, inhalation and ingestion of contaminated food.

Table 4. Comparison of Estimated Dose Rate to Man From ^{233}U Fuel vs Pu Fuel Assuming 10^{-12} g/sec Release Rate

Exposure Pathway or Organ	^{233}U Fuel Dose Rate (mrem/year)	Percent from ^{232}U	Pu Fuel Dose Rate (mrem/year)	U/Pu Dose Rate Ratio
Submersion in Air				
Total Body	1.5×10^{-4}	92	7.3×10^{-7}	2.1×10^2
Ground Exposure				
Total Body	2.0×10^{-3}	92	1.2×10^{-4}	1.7×10^2
Inhalation				
Total Body	3.6×10^{-1}	65	9.2×10^1	3.9×10^{-3}
Bone	6.9×10^0	48	3.8×10^3	1.8×10^{-3}
Ingestion				
Total Body	6.7×10^{-1}	94	5.7×10^{-1}	1.2×10^0
Bone	9.7×10^0	93	2.4×10^1	4.0×10^{-1}

Several aspects of the data in Table 4 are important and should be summarized. First, when all pathways are considered, the plutonium fuel presents a greater dose than uranium fuel by a factor of approximately 500. Bone is the critical organ for both fuels. Second, if the ingestion pathway is the critical pathway, as in the case of a facility no longer releasing fuel to the atmosphere, both fuels have similar radiological impact. Finally, it is significant that ^{232}U is the primary contributor to the total dose from uranium fuel for each organ.

The TERMOD computer code estimates radionuclide intake to man through ingestion of milk, beef, and crops. This code indicated that uranium moves through the food chain at a greater rate than plutonium.

From these data it was concluded that high specific activity uranium, particularly ^{232}U , must be regarded as having high radiotoxicity. It was also concluded that a more thorough evaluation of the radiological impact of ^{232}U and daughters in HTGR fuel was needed.

A follow up study of ^{232}U and daughters was completed which focused on three principal areas:^{17,18} first, an assessment was made of the radiological impact of ^{232}U and daughters relative to the other radionuclides which would comprise the airborne releases from a hypothetical HTGR fuel reprocessing plant; second, the effect on the 50-year dose commitment to bone from increasing the concentration of ^{232}U in recycled ^{233}U HTGR fuel was reviewed; third, the impact of ^{232}U daughter buildup in the environment on the dose commitment to bone resulting from inhalation or ingestion of equal masses of LWR uranium fuel, HTGR uranium fuel, and LMFBR plutonium fuel was evaluated. Because the first two

areas are directly related to this study, they are reviewed in the following paragraphs.

The radiological impact was calculated for ^{232}U and daughters in addition to fission products and transuranium elements released from a hypothetical HTGR fuel reprocessing facility. With the exception of ^{14}C , theoretical source terms were computed using curies per metric ton heavy metal (Ci/MTHM) inventories at 150 days after removal from a reactor as reported by Blomeke et al.²⁹ The ^{14}C source term was assumed to be 5.0×10^3 Ci/year. The model plant reprocesses 450 metric tons of heavy metal annually. Therefore the source term corresponds to 11.1 Ci/MTHM if one assumes a decontamination factor of one for ^{14}C .³⁰ Carbon-14 is assumed to be released as $^{14}\text{CO}_2$.

Table 5 lists the 50-year dose commitments in mrem to total body, bone, and lungs for particulates. Doses are shown in decreasing order of magnitude. The only significant source for the particulates ^{228}Th and ^{224}Ra in the source term is the decay of ^{232}U ; consequently, these three radionuclides are combined into a single dose. This dose is listed in Table 5 as $^{232}\text{U}^*$. The dose contribution from ^{232}U particulate daughters other than ^{228}Th and ^{224}Ra is insignificant.

Uranium-232* contributes 10% of the dose to the lungs, 4% of the dose to the bone, and 4% of the dose to the total body. Although $^{232}\text{U}^*$ ranks high in Table 5 (3rd for lungs, 4th for total body, 6th for bone), it is concluded that effluent control sufficient to keep doses from Sr and Cs at acceptable levels also provides satisfactory treatment for ^{232}U and particulate daughters. However, the high

Table 5. Ranking of Significant Particulate Radionuclides By Dose^a to Reference Organs at 1.5 Miles from Stack

Total Body		Bone		Lungs	
Radionuclide	Dose (mrem)	Radionuclide	Dose (mrem)	Radionuclide	Dose (mrem)
^{134}Cs	1.40	^{90}Sr	9.8	^{137}Cs	0.70
^{137}Cs	0.93	^{137}Cs	1.3	^{134}Cs	0.62
^{90}Sr	0.20	^{134}Cs	1.2	$^{232}\text{U}^{\text{b}}$	0.20
$^{232}\text{U}^{\text{b}}$	0.11	^{238}Pu	0.9	^{90}Sr	0.20
^{106}Ru	0.08	^{244}Cm	0.9	^{244}Cm	0.05
^{244}Cm	0.05	$^{232}\text{U}^{\text{b}}$	0.6	^{154}Eu	0.04
Others	0.16	Others	0.7	Others	0.15
Total	2.93		15.4		1.96

^aDoses are 50-year dose commitments.

^bThe dose from ^{232}U includes the dose from ^{228}Th and ^{224}Ra daughters.

position of $^{232}\text{U}^*$ in Table 5 indicates potential significance for exposure from these particulates. The position of $^{232}\text{U}^*$ also suggests that a more thorough evaluation of these radionuclides, especially long-term effects, may be necessary.

A breakdown of percentage of the dose from each component in $^{232}\text{U}^*$ is summarized as follows. The dose to total body is due almost entirely to exposure from ^{232}U (50%) and ^{224}Ra (45%). Radium-224 contributes 64% of the dose to bone; ^{232}U , 20%; and ^{228}Th , 16%. The dose to lung is more evenly divided between ^{232}U (35.3%), ^{228}Th (37.5%) and ^{224}Ra (27.2%).

The radiological impact for volatile radionuclides at a distance of 1.5 miles is summarized in Table 6. Doses are listed in decreasing order of magnitude. Radon-220 accounts for only 0.3% of the dose to

Table 6. Ranking of Significant Volatile Radionuclides by Dose^a to Reference Organs at 1.5 Miles from Stack

Total Body		Bone		Lungs	
Radionuclide	Dose (mrem)	Radionuclide	Dose (mrem)	Radionuclide	Dose (mrem)
^3H	16.0	^{14}C	16.0	^3H	16.0
^{14}C	12.0	^3H	11.0	^{85}Kr	16.0
^{85}Kr	7.7	^{85}Kr	9.4	^{14}C	5.2
^{129}I	2.2	^{129}I	2.8	^{220}Rn	2.1
^{131}I	0.7	^{220}Rn	2.1	^{129}I	1.4
^{220}Rn	0.3	^{131}I	0.8	^{131}I	0.6
Total	39.2		42.1		41.3

^aDoses are 50-year dose commitments.

the lungs. In each case, more than 99.9% of the ^{220}Rn dose results from inhalation of the daughter, ^{212}Pb .

Figure 3 shows the effect of increasing the concentration of ^{232}U in recycled ^{233}U HTGR fuel on the 50-year dose commitment to bone. The dose commitment is calculated assuming 10^{-12} g of fuel that has been separated at a reprocessing plant is inhaled. The lower curve is the dose commitment for HTGR fuel at 90 days following separation. The upper curve is the dose commitment for HTGR fuel at 10 years following separation. The broken horizontal line near the top of the graph indicates the dose commitment from inhalation of 10^{-12} g of freshly separated LMFBR fuel; the lower broken line is dose from inhalation of 10^{-12} g of ^{233}U fuel with no ^{232}U . The vertical broken line marks the maximum anticipated ^{232}U concentration of 1200 ppm at equilibrium recycle.

Increasing the concentration of ^{232}U from 0 ppm to 1200 ppm causes the 50-year dose commitment to bone to increase by a factor of approximately 35 for 90 day-old fuel and a factor of approximately 185 for fuel with ^{232}U and daughters at equilibrium. Two basic conclusions may be drawn from this figure. First, as buildup to equilibrium recycle develops and concentration of ^{232}U become greater, overall radiotoxicity of ^{233}U HTGR fuel increases significantly. Second, the buildup of ^{232}U daughters in ^{233}U fuel also increases fuel toxicity significantly for a given concentration of ^{232}U .

The significance of ^{232}U in HTGR fuel and in other materials has been reported elsewhere in the literature. Wrenn³¹ also studied the potential relative toxicities of uranium and plutonium in LWR and HTGR

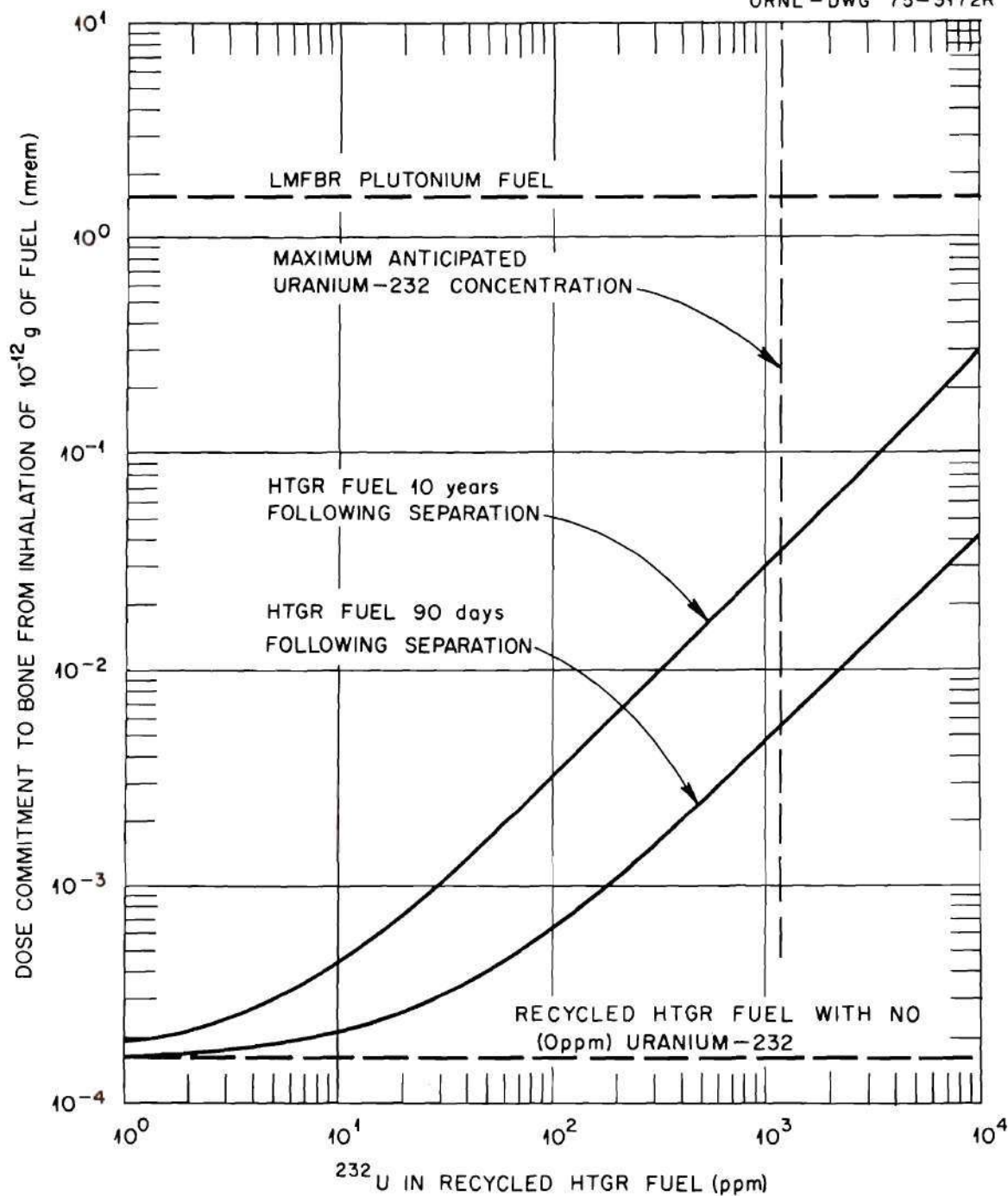


Figure 3. Effect of ^{232}U Concentration in ^{233}U Fuel on Dose Commitment to Bone

fuel. Although that study was not a comprehensive analysis, it concluded that ^{233}U HTGR fuel with moderate concentrations of ^{232}U , from a toxicological standpoint, represents a new more toxic type of uranium.

External exposure from gamma radiation emitted by the ^{232}U daughters ^{212}Pb , ^{212}Bi , and ^{208}Tl has been reviewed by Arnold³² for in-plant operations. Buildup of ^{232}U daughters in ^{233}U fuel following separation at the reprocessing plant creates special handling problems for the next step in the fuel cycle, fabrication into elements. Exposures can be controlled by fabricating the elements soon after separation or by incorporating remote fuel handling equipment into the facility design. It has been demonstrated³³ that ^{232}U in recycled LWR fuel may lead to increased levels of exposure during the gaseous diffusion process although these exposures still do not present significant health hazards.

In view of the preceding discussion, it was anticipated that the present research to determine the toxicity of high specific activity uranium to one type of aquatic organism, the developing fish egg, would contribute useful data to the total assessment of the thorium fuel cycle.

Review of the Literature

This literature review focuses on experimental data which describe the effects of ionizing radiation on the developing embryos of fish. The particular radionuclide used, the mode of exposure, and the experimental technique significantly affect the outcome of each study. Therefore this review is divided into the following areas:

effect of radiation on all aquatic biota; exposure from external penetrating radiation; exposure during development in radioactive solutions; and exposure to uranium or plutonium. A summary of the experimental data concludes this review.

Several publications in the literature review the effects of ionizing radiation on aquatic organisms. Polikarpov^{34,35} has provided extensive, well organized, and well documented reviews. Ophel et al.³⁶ prepared a summary of the effects of ionizing radiation on aquatic biota and made several specific recommendations for future research needs. Templeton et al.^{37,38} have reviewed experimental studies which provided an assessment of the dose to aquatic organisms and extracted data from the literature on contamination of the aquatic environment. Using this information Templeton predicted doses to aquatic biota exposed to radionuclides found in nature. Woodhead³⁹ assessed the dose to developing fish eggs due to the accumulation of radioactivity. A dosimetry model was presented in his paper which calculated the dose to eggs exposed to radioactive wastes which are disposed in the ocean. More recently, Kaye²⁶ and Blaylock and Witherspoon⁴⁰ have discussed the dose to aquatic biota exposed to radionuclides released in the nuclear fuel cycle and predicted that there would be no significant effects from this source; it was noted, however, that there was a lack of information in the literature on the effects of low level exposures.

Exposures from External Penetrating Radiation

Chronic Exposure. Brown and Templeton⁴¹ and Templeton⁴² exposed the eggs of brown trout (*Salmo trutta*) and plaice (*Pleuronectes platessa*)

to external gamma radiation at dose rates ranging from 0.035 R/day to 150 R/day. These experiments failed to demonstrate a significant reduction in hatching or the occurrence of abnormal larvae. However, the use of antibiotics and a low temperature regime may have created a protective mechanism which affected the outcome of this study. A similar experiment by Donaldson and Bonham⁴³ in which eggs of chinook salmon (*Oncorhynchus tshawytscha*) and coho salmon (*O. kisutch*) were exposed to 0.5 R/day of ⁶⁰Co gamma radiation resulted in a significant difference between irradiated eggs and controls for opercular defects. Other indicators of radiation damage such as survival, growth, vertebral numbers, and sex ratios were observed although no discernible differences could be demonstrated. In another chronic exposure experiment, Bonham and Donaldson⁴⁴ exposed chinook salmon (*Oncorhynchus tshawytscha*) to dose rates of 0.5 R/day to 50 R/day during their 2.5 month development period. A dose rate of 10 R/day markedly inhibited gonadal development up to the migratory stage. Bonham and Donaldson used a large number of fish and observed irradiated offspring over several generations. Abnormalities of offspring were increased by increasing the dose rate; low dose rates appeared to have a beneficial effect as measured by a greater number of irradiated stock returning to spawn than in the control stock. These long-term studies are very significant and incorporate observations of genetic as well as somatic damage.

Acute Exposure. Most experiments evaluating the effects of radiation on the eggs of fish or aquatic organisms have used acute exposure rather than chronic exposure. Many studies have dealt with the sensitivity

of embryos at different stages of development. Welander⁴⁵ exposed the eggs of rainbow trout (*Salmo gairdneri*) to x rays during different embryonic stages of development. Doses of 25 R to 2570 R were used. Mortalities, abnormalities, and retardation in growth were observed among the hatched fry. The one-cell stage of development was determined to have the highest radiosensitivity. Doses of 300 R to 900 R were reported and LD₅₀ values for the five other stages evaluated. A noticeable retardation of growth and length was reported after 38 R exposure during the early-eyed stage. A more comprehensive follow-up study on this experiment yielded similar conclusions.⁴⁶

McGregor and Newcombe⁴⁷ irradiated the germ cells of trout (*Salmo gairdneri*) to acute gamma radiation at doses of 25 rads to 400 rads. A decreased mortality was noted with the lower doses indicating that a beneficial effect from radiation may exist. Doses from 200 rads to 400 rads resulted in increased mortality of the eggs. The harmful effect to the sperm at higher doses was presumed to be gross chromosomal rearrangements; a valid explanation was not offered for the beneficial effect of lower doses. Newcombe⁴⁸ reviewed this same experiment and emphasized the need for caution before equating low doses of radiation to human germ cells with apparent increase in fetal and infant mortality.

Adult rainbow trout (*Salmo gairdneri*) were irradiated with x rays at total-body doses of 50 R to 2500 R by Foster et al.⁴⁹ The eggs and larvae were observed for mortalities and abnormalities. Abnormalities increased with dose and survival of larvae decreased with

the amount of exposure. During the fry stage of development mortality in every exposure group was significantly higher. This report included information and photographs which described radiation-induced abnormalities in young larvae.

Rugh and Clugston⁵⁰ studied the effects of various levels of x radiation on the gametes and early embryos of *Fundulus heteroclitus*. Exposure levels of 1000 R-200,000 R of 182 kVp x rays at an exposure rate of 6000 R/min were used for the experiment. Eggs were irradiated both before and after fertilization. Those irradiated prior to fertilization did not develop fully, but exposures less than 50,000 R were not evaluated. Eggs irradiated during the 1-2 cell stage of development were the most sensitive. Under exposures of 300 R-400 R, egg development was retarded. In another study using *Fundulus heteroclitus* embryos, Solberg⁵¹ demonstrated that eggs exposed to a dose of 2,000 rads several hours after fertilization produced 100% deformed larvae. This experiment also evaluated changes in radiosensitivity with mitotic index. Solberg's experiment of radiation-induced damage to fish embryos was among the earliest work reported in the literature of this type.

Bonham and Welander⁵² demonstrated the increase in radioresistance of fish eggs with advancing embryonic development using the eggs of silver salmon (*Oncorhynchus kisutch*). Twenty-three stages of development from zygote to late-eyed stage were exposed to doses ranging from 12 R to 2,400 R. It was concluded from this research that sensitivity of the embryo to ionizing radiation decreases with advancing

development. An $LD_{50/150}$ as low as 16 R was shown for the early one-cell development stage. However, these scientists were not able to show an association of radiosensitivity with mitotic index. Chinook salmon eggs (*Oncorhynchus tshawytscha*) were exposed to x rays at four developmental stages in duplicate experiments, one at ambient temperature and one at two degrees ($^{\circ}C$) above ambient temperature.⁵³ An exposure of 15 R during the one-cell stage at the higher temperature produced a significant increase in mortality. This study indicated the existence of greater radiosensitivity at earlier stages of development and at higher temperatures.

Several papers by Kulikov have reported the effect of ^{60}Co gamma radiation on the embryos of tench (*Tinca tinca*). In the first experiment⁵⁴ a single external irradiation of the roe during early cleavage was used to demonstrate an LD_{100} at hatch of 400 R. In addition, morphological anomalies were appreciably increased at 50 R for embryos irradiated at the first cleavage. In a second study⁵⁵ using the eggs of tench (*Tinca tinca*) it was demonstrated that eggs receiving 25 R to 50 R of ^{60}Co radiation during early cleavage followed by 4 kR of supplementary radiation after hatching showed a greater survival rate than controls. The conclusion of this report suggested the presence of a beneficial effect of low doses of radiation. Kulikov⁵⁶ again exposed tench (*Tinca tinca*) to ^{60}Co radiation during different developmental stages and observed the effect on time of hatch, number hatching, and abnormalities. Based on the number of larvae hatching, this experiment showed the most sensitive stage to ionizing radiation

to be before the first cell division. Prelarvae that hatched from eggs irradiated at an exposure of 250 R during early cleavage had an increased mortality rate during the development period after hatch.

Belyaeva and Pokrovskaya⁵⁷ exposed embryos of loach (*Misgurnis fossilis*) to external gamma radiation during several developmental stages. Sensitivity was established by percentage of chromosomal rearrangements, percentage of mortalities, and percentage of abnormalities. It was reported that an exposure as low as 50 R at the time of greatest sensitivity was sufficient to cause 30% mortality and produce a statistically higher number of abnormalities. Allen and Mulkey⁵⁸ conducted a similar experiment using the eggs of the paradise fish (*Macropodus opercularis*). The eggs were exposed to x rays at an exposure dose of 1000 R during developmental stages. This level of exposure was sufficient to cause a suppression of mitotic activity. A dose of 1000 R during the first six hours of development prevented hatching in 99% of the eggs.

The effect of fractionated doses of x rays on the eggs of *Fundulus* was studied by White.⁵⁹ Embryos were allowed to develop to the 2-16 cell stage before being irradiated with 100 kVp x rays. Fractionated exposures were administered once every 24 hours at levels of 100 R, 250 R, 500 R, 1000 R, and 2000 R. The criteria for radiation effects were the rate of development of each egg, survival of the embryo, and hatching. All doses produced a retardation in growth. No observable effects were noted on embryos irradiated at 100 R/day

while exposures greater than this caused an appreciable reduction in the number hatching.

Another study demonstrating the variation in sensitivity of embryos with time of exposure was conducted by Frank.⁶⁰ Eggs of carp (*Cyprinus carpio*) were fertilized in the laboratory and exposed to acute gamma radiation at levels of 500 rads to 16,000 rads. Figure 4 summarizes the findings in this study. A dose of 16,000 rads at 24 hours did not interfere with hatching although 4000 rads or more caused gross abnormalities in most larvae. At 40 hours 16,000 rads had no effect on hatchability; no significant increase in the number of abnormal larvae was noted. The most radiosensitive stage of development was found to be 30 minutes after fertilization when the eggs were in the zygote stage. A dose of 500 rads at this time caused a reduced number of embryos to hatch. In another study using carp (*Cyprinus carpio*), Blaylock and Griffith⁶¹ reported that when eggs were exposed to 500 rads 30 minutes after fertilization, a significant reduction was noted in the number of embryos that hatched. A dose of 1000 rads at that stage prevented hatching of the eggs.

Very little information is found in the literature which reviews the genetic and cytogenetic effects of radiation on eggs of fish. In a study using irradiated eggs of *Oryzias latipes*, Egami and Hyodo-Taguchi⁶² observed abnormalities in the gonads of fish that had been irradiated at eight different stages of development with doses of 0.5 kR to 2 kR of x rays. A greater number of sterile testes were observed in fish which had been irradiated 7-8 days after fertilization

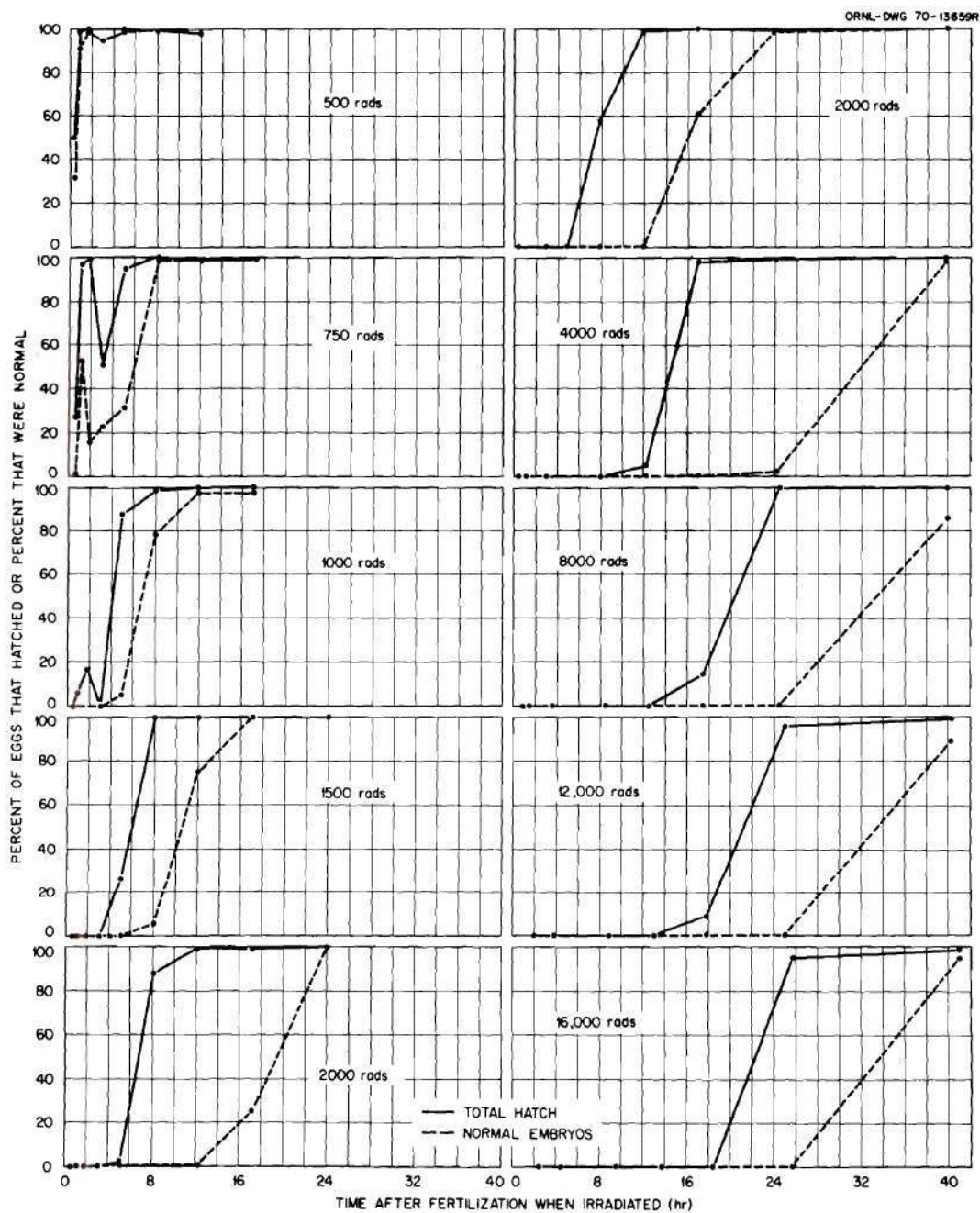


Figure 4. Effect of ^{60}Co Gamma Radiation on Eggs of Carp Irradiated at Different Stages of Development (Frank⁹⁵)

than those irradiated 1-3 days after fertilization. Woodhead⁶³ observed chromosome aberrations in irradiated embryonic tissue of *Ameioba splendens* and obtained a dose-response curve. He noted that chromosome damage was similar to that found in mammalian systems.

A more general study of the effects of radiation on the embryos of fish by Romashov et al.⁶⁴ evaluated three principal areas: whole body radiation damage to the gonads of carp (*Cyprinus carpio*); diploid radiation gynogenesis; and persistence of radiation damage to chromosomes in embryonic development. This study concluded that fish display stages of radiation injury known for other vertebrates; however, lethal doses are higher.

Only two experiments reported in the literature evaluated the effects of external radiation other than gamma or x rays. Hyodo-Taguchi et al.⁶⁵ exposed embryos of *Oryzias latipes* at four developmental stages to various doses of 2 MeV fast neutrons and 200 kVp x rays. Hatchability, mortality, and abnormalities were observed. Doses ranged from 100 rads to 8000 rads for neutron and 15 rads to 7600 rads for x rays. The culture medium, changed daily, contained 2000 ppm penicillin. The neutron exposures produced a significant effect on hatchability at 225 rads when the embryos were irradiated during the 32 cell stage. More abnormalities were noted with fast neutrons than x rays when the embryos were irradiated during the germ-ring stage (30 hours after fertilization) and at the beginning of blood circulation (80 hours after fertilization). Because two types of radiation were used in the experiment, values for relative

biological effectiveness (RBE) were calculated using median hatchability for a comparison of effectiveness. Values of RBE of neutrons for each stage were reported as follows: stage I (5 hours), 5.6; stage II (30 hours), 6.0; stage III (80 hours), 7.1; and stage IV (6 days), 1.7.

Blaylock et al.⁶¹ exposed carp eggs (*Cyprinus carpio*) to acute beta radiation using a plane disk source that contained 750 mCi of ^{90}Sr - ^{90}Y . The dose rate to the eggs was calculated to be 1.4 rads/second and irradiation was performed 30 minutes after fertilization. A significant effect, based on percent hatch, was observed at a dose of 370 rads. Simultaneous exposures were also conducted with gamma radiation and although no quantitative RBE value was established, effects were noted at lower doses of beta radiation than gamma radiation.

Exposure During Development in Radioactive Solutions

A number of experiments are reported in the literature in which fish eggs were developed in concentrations of radioactive materials. Templeton⁴² and Brown and Templeton⁴¹ exposed eggs of brown trout (*Salmo trutta*) and plaice (*Pleuronectes platessa*) to ^{90}Sr - ^{90}Y over concentrations ranging from 10^{-7} $\mu\text{Ci/ml}$ to 10^{-1} $\mu\text{Ci/ml}$. Eggs were placed into the solution immediately after fertilization; hatchability, abnormal larvae, and larval length were observed. The data indicated that neither a consistent reduction in hatchings nor an increase in the number of abnormal larvae could be demonstrated. During these experiments antibiotics added to the water in which the plaice were developing may have affected the sorptive properties of the eggs and provided an artificial protective mechanism. Strand et al.⁶⁶ exposed

trout embryos (*Salmo gairdneri*) to tritiated water during embryogenesis and observed an immune response during development and increased susceptibility of the larvae to disease following irradiation.

Fedorov⁶⁷ evaluated the effects of radioactive contamination on the later stages of embryonic development using eggs of salmon at the sixth stage of development (approximately seven days prior to hatching). Roe were placed in solutions of ⁹⁰Sr at concentrations of 3×10^{-7} $\mu\text{Ci/ml}$ to 5×10^{-6} $\mu\text{Ci/ml}$, and ¹³⁷Cs at concentrations ranging from 6×10^{-7} $\mu\text{Ci/ml}$ to 2.5×10^{-6} $\mu\text{Ci/ml}$. In this study Fedorov concluded that even in late stages of development, roe were susceptible to radiation damage. Contradictory results were reported by Templeton⁴² and by Kulikov et al.⁵⁴ who used ⁹⁰Sr solutions having a greater concentration of activity; no significant effects could be determined.

An excellent experiment was reported by Fedorova⁶⁸ in which the eggs and developing larvae of crucian carp (*Carassius carassius*), the roach (*Rutilus rutilus*), alburnum (*Alburnus alburnus*) and striped perch (*Acerina cernua*) were used to study the effects of ¹⁴C as $\text{CH}_3^{14}\text{COOH}$. The ¹⁴C activity ranged from 2×10^{-3} $\mu\text{Ci/ml}$ to 2×10^{-1} $\mu\text{Ci/ml}$. Embryos were placed in solutions at the early cleavage stage (8-16 blastomeres) and larvae were left to develop in the contaminated water. Viability of ova, hatching speed, radioactivity of the larvae, and abnormalities were measured. A significantly greater number of egg mortalities was noted in solutions of 2×10^{-2} $\mu\text{Ci/ml}$ to 2×10^{-1} $\mu\text{Ci/ml}$. Hatching speed of ¹⁴C contaminated eggs was significantly less than controls. Abnormal larvae were observed in alburnum, striped perch,

and roach. Although the activity concentration was greater than that used by Polikarpov and Ivanov⁶⁹ and Fedorova⁶⁸ and fission products investigated by Mikami et al.,⁷⁰ deaths and abnormalities were significantly less with ^{14}C . This difference was attributed to the lower energy of the beta radiation from ^{14}C and also suggested that the absorbed energy depends on the isotope used.

Polikarpov and Ivanov⁶⁹ reported effects on developing kamsa spawn and rock bass. Hatchability, growth retardation, and abnormalities resulting from ^{90}Sr (2×10^{-5} $\mu\text{Ci/ml}$ to 0.2 $\mu\text{Ci/ml}$) were observed. Abnormalities fell into two groups: vertebral, or bending of the spine; and teratological effects including cyclopean or acephalic effects and pigmentation of the eyes. A diagram showing the percentage of spawn which were abnormal, and the percentage dying in the early stages of development indicated that the most sensitive effects observed were abnormalities.

Ivanov⁷¹ conducted experiments on various species using several different radionuclides. Accumulation factors were determined for ^{89}Sr , ^{90}Sr - ^{90}Y , ^{91}Y , ^{106}Ru , ^{137}Cs , ^{144}Ce , ^{59}Fe , and ^{35}S . Radionuclides were divided into two groups, those that accumulate on the egg surface (Y, Ce, Fe), and those that localize in the tissue of the embryo (Sr, Cs). Ivanov⁷² also used the eggs of artificially fertilized marine fish to calculate the accumulation factor (ratio of the concentration in eggs to water) for the following radionuclides; ^{14}C , ^{32}P , ^{35}S , ^{54}Mn , ^{59}Fe , ^{60}Co , ^{89}Sr , ^{90}Sr , ^{90}Y , ^{91}Y , ^{95}Zr , ^{95}Nb , ^{106}Ru , ^{137}Cs , ^{144}Ce , and ^{185}W . In this study Ivanov concluded that most of the activity was associated

with the egg membrane rather than the egg contents. Adsorption of radionuclides on the membrane was assumed to be independent of the activity in the cell and also independent of the type of egg being used. It was suggested that the mechanism of uptake of radionuclides by embryos is due to passive diffusion through the membrane. Finally, Ivanov concluded that uncharged complexes permeate the egg membrane much more readily than charged complexes. Therefore the degree of permeation may be controlled by the degree of hydrolysis or polymerization in the environment.

Tsytsugina⁷³ investigated chromosome breaks during anaphase and telophase in eggs of ruff (*Scorpaena porcus*) developing in solutions containing ^{90}Sr - ^{90}Y . It was concluded that concentrations as low as 10^{-6} $\mu\text{Ci/ml}$ produced more breaks than were observed in control solutions.

Most of the experiments conducted by Russian scientists lead to the conclusion that eggs of fishes are extremely sensitive to exposure from radiation. This high sensitivity has generally not been observed by other experimenters. Trabalka⁷⁴ repeated the experiments conducted by Polikarpov and Ivanov⁷⁵ using a different methodology in an attempt to observe the effects of low concentrations of ^{144}Ce - ^{144}Pr on developing eggs. In his study Trabalka developed the eggs of fat-head minnows (*Pimephales promelas*) in ^{144}Ce - ^{144}Pr at a concentration of 1.2×10^{-4} $\mu\text{Ci/ml}$. No statistically significant differences between the treated and control groups could be demonstrated. This experiment was conducted under carefully controlled environmental conditions in which an entire community of organisms was exposed.

Exposure to Uranium or Plutonium

There have been no data reported in the literature on the effects that uranium has on developing fish eggs. Several experiments have been performed with plutonium; however, there is no information on the uptake of plutonium by the egg contents.

The most extensive investigation of plutonium was reported by Patin et al.⁷⁶ In this experiment the accumulation of $^{239}\text{Pu}(\text{NO}_3)_4$ by live and dead *Misgurnis fossilis* spawn was studied. Eggs were developed in solutions containing $^{239}\text{Pu}(\text{NO}_3)_4$ at concentrations of 9×10^{-5} $\mu\text{Ci/ml}$ and 9×10^{-4} $\mu\text{Ci/ml}$. Patin suggested that the accumulation of plutonium was related to two sets of factors; the physiochemical form in which plutonium was present in the solution and the physiological state of the egg. Also, it was concluded that in view of the impermeability of the spawn membrane to components in the water medium, especially colloidal particles, the plutonium was most likely concentrated on the egg membrane. Dead *Misgurnis fossilis* spawn accumulated activity with no tendency to saturate. Finally, Patin demonstrated that ^{239}Pu was eliminated from live spawn, primarily during the first 10 hours after they were transferred to an uncontaminated solution. Although the data presented in that paper were useful in terms of bioaccumulation of ^{239}Pu on fish eggs, it was of limited use in providing any quantitative information on the distribution of plutonium inside the egg which is needed for assessment of the radiological dose.

Auerbach et al.⁷⁷ reported the results of some preliminary studies to evaluate the effects of ^{239}Pu on carp (*Cyprinus carpio*)

eggs. At concentrations ranging from 5×10^{-4} $\mu\text{Ci/ml}$ to 5×10^{-1} $\mu\text{Ci/ml}$ no observable effects could be seen as determined by hatching or abnormal frequency. Woodhead⁷⁸ estimated doses to the eggs of plaice (*Pleuronectes platessa*) from actinide wastes which were released into the ocean. Two different whole egg concentration factors were calculated under identical laboratory conditions. In the first case a concentration factor of 5.8 at the time of hatching was observed, and in the second case a concentration factor of 35 was reported at the time of hatching. It was suggested that the large difference between these two concentration factors was due to the phenomenon of surface adsorption of the ^{239}Pu on the egg membrane. Another possible explanation offered for the higher accumulation factor in the second experiment was that more bacteria were present.

Summary

This review of the literature leads to several conclusions about knowledge of the effects of radiation on developing fish eggs. In general some good data are available that discuss the effects of beta and gamma radioactivity. There is almost no information on the effects of alpha emitting radionuclides and, in particular, plutonium or uranium.

The dose for which effects are first seen appears to vary considerably among studies in the type of radiation used, and whether the exposure was from an external source or whether radioactive material was added to the solution in which the eggs were developing. The consensus seems to be that eggs are most sensitive during the period of

time soon after fertilization and that radiosensitivity decreases as development proceeds.

With the exception of two papers by Ivanov^{71,72} and one experiment by Woodhead,³⁹ no attempts have been made to evaluate the penetration of the egg membrane by radionuclides. There are no quantitative data to show the fraction of activity associated with the egg contents and the membrane or the distribution of alpha emitting radionuclides incorporated into the egg contents. This information is particularly important for assessment of the dose from alpha radiation as well as from low-energy beta or gamma radiation. Often no attempt was made to evaluate the absorbed dose and results were simply reported as observed effects as a function of solution concentration.

It was concluded from this review that studies on the effects of plutonium and uranium on developing fish eggs would contribute significantly to the knowledge and understanding of the impact these materials have on certain biota in the environment.

Purpose of This Research

This research project has consisted of three principal tasks.

1. To determine the behavior of plutonium and uranium in solution when associated with the developing embryos of fish.
2. To determine the radiological and chemical toxicity of plutonium and uranium to developing embryos of fish.

3. To assess the impact which plutonium and uranium found in the natural environment may have on developing embryos of fish.

Prerequisites for the study of the behavior of plutonium and uranium associated with the developing embryos of fish include the following areas of research; the penetration of plutonium and uranium through the chorion, the concentration and distribution of plutonium and uranium in the egg contents, the stage of development at which uptake occurs, the distribution of these elements in the egg volume, and the dose received by sensitive tissue within the developing egg.

A study of the state-of-the-art recommended testing for environmental transport of toxic substances suggested that the hierarchy for specific testing follow determinations for toxicity, persistence, and dispersion.⁷⁹ In order to determine the radiological and chemical toxicity of plutonium and uranium to developing embryos of fish, it was necessary to observe several specific effects that are caused by radioactive or chemical toxicants. Effects on hatchability, abnormalities, and larval survival were observed to establish limits of toxicity. The chemical toxicity of plutonium was of particular interest in this study because scientists have not been able to demonstrate conclusively the chemical toxicity of this element. Investigators have reported a difference between morphological, histopathological, and hematological factors which were produced when mammals were injected with equal microcurie quantities of ^{238}Pu and ^{239}Pu .^{80,81} This difference has been explained by the mass distribution of ^{238}Pu and ^{239}Pu ; it has

also been suggested that chemical toxicity may also contribute to the difference⁸² since equal activities imply approximately 3000 times the quantity of plutonium for ^{239}Pu compared with ^{238}Pu .

The third objective in this study presents a practical application of the experimental and theoretical findings on the biochemical behavior and toxicity of plutonium and uranium to developing embryos of fish. This objective is accomplished using data reported in the literature on controlled and uncontrolled releases of plutonium and uranium to the aquatic environment.

CHAPTER II

CHARACTERISTICS OF THE TEST SYSTEM

Fish Eggs as an Aquatic Test Organism*Cyprinus carpio* (Linnaeus)

Reproductive Biology/Embryology. The feasibility of using eggs of *Cyprinus carpio* (Linnaeus), as a biological test system was first reported in the literature by Blaylock.⁸³ The percentage of hatch reported by Blaylock was approximately 97% for 28,762 eggs that were fertilized. Carp larvae have also been employed for toxicological studies; the behavior of the larvae in a contaminated stream was observed.⁸⁴ However, such a toxicity test is not adaptable to studies of plutonium and uranium due to the large experimental apparatus that is needed and the hazards that are involved in working with radioactive isotopes of these elements.

The reproductive biology of *C. carpio* has been thoroughly studied and is well documented in the literature.^{85,86,87,88} The male carp has a one-year sexual cycle which may be divided into five periods, each period being characterized by a typical change in the testes:⁸⁷

1. The period just before spawning (4-5 weeks)
2. The period of spawning (2-6 weeks)
3. The period of regeneration (2-3 weeks)
4. The period of active spermatogenesis (5-6 months)
5. The period of slow spermatogenesis (2-3 months)

In eastern Tennessee, the first period occurs during March or April. The initiation and the duration of the spawning period depend on weather and spawning ground conditions, i.e., lake levels, turbidity of water, and water temperature. Any change in these factors may interrupt and delay spawning. During the spawning period, milt is discharged from the males when very light pressure is exerted on the abdominal walls. Males usually become sexually mature during their third or fourth year of age and rarely maintain sexual maturity over ten years of age.

The sexual cycle of female carp also lasts one year. Oogenesis may be divided into five periods which are characterized by a change in the ovaries:⁸⁷

1. The period just before spawning (3-4 weeks)
2. The period of spawning (2-6 weeks)
3. The period of regeneration (up to 3 months)
4. The period of active oogenesis (3-4 months)
5. The period of slow oogenesis (up to 3 months)

As with the males, the period just before spawning usually occurs during March or April and the spawning period is affected significantly by environmental conditions. Females reach sexual maturity approximately one year later than males. During the spawning period, eggs can be extracted from the female by lightly stroking the abdomen. Sexual dimorphism for females or males is not evident by external examination except during the spawning season.

Fecundity as related to length, weight, and age of female carp has been studied in Lake St. Lawrence, Ontario.⁸⁵ Among the specimens

examined, egg production varied from 36,000 eggs in a three-year-old fish of 394 mm length to 2,208,000 eggs in a sixteen-year-old fish with a length of 851 mm. The age composition and fecundity for spawning carp on Lake St. Lawrence are illustrated in Figure 5.

Spawning carp are abundant in eastern Tennessee during spring. As temperatures in shallow grassy embayments begin to exceed approximately 18° C, carp begin to migrate from the deeper waters. The sex ratio of males to females in spawning areas is reported to be 1.8:1.⁸⁵ During spawning, the female is usually followed into shallow areas by one or more males. Although the most active spawning was observed in flooded grassy shores and inlets, spawning occurred in embayments which contained floating wood and other debris. Spawning was also reported in an open deep water area of Watts Bar Lake on several occasions.⁸⁹ Maximum spawning activity was seen when water temperatures exceeded 22° C.

Females are stimulated by males as the fish swim together and both males and females move their tails violently splashing the water. The eggs are released and are then fertilized by milt. The time of day which spawning occurs depends on environmental conditions such as overnight temperature, rainfall, and lake levels. During the peak of the spawning period, activity was observed from sunrise until sunset.

Because the ratio of males to females during spawning is nearly 2:1, males are much easier to collect than females. In addition, females usually expend their eggs in a shorter period than males expend their sperm. After a female has completed spawning she leaves the area; the male then may seek another ripe female.

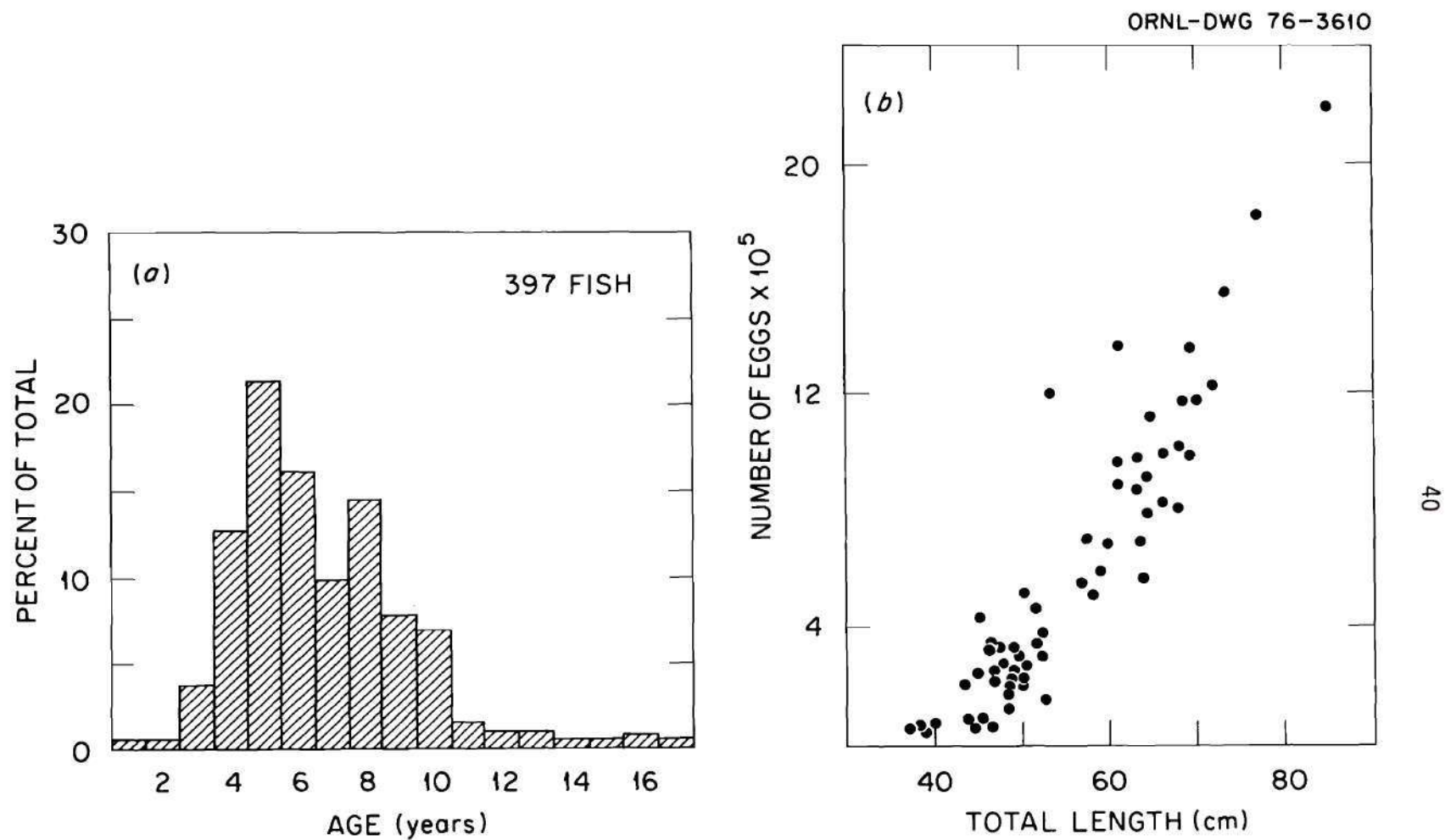


Figure 5. Typical Age Distribution of Adult Carp and the Relationship Between Fecundity and Total Length of Female Carp on Lake St. Lawrence (Swee and McCrimmon⁸⁵)

If water conditions and water levels are favorable, the duration of the spawning period may extend over several months. Spawning was observed in the lakes with warmer water temperatures first. The normal sequence of spawning in the Oak Ridge National Laboratory area is Watts Bar Lake, Fort Loudon Lake, Cherokee Lake, Norris Lake, Douglas Lake, Melton Hill Lake and the Clinch River above Melton Hill Lake. In 1975, spawning began in Watts Bar Lake on April 25, 1975 and extended through June. The peak period occurred during May. After this time it became increasingly difficult to collect ripe specimens.

The embryological development of the *C. carpio* egg and the sensitivity of *C. carpio* eggs to varying environmental conditions are well documented in the literature.^{90,91,92,93} Because carp are not used as a primary source of food in the United States, most of the literature about carp rearing and production comes from foreign countries, particularly in Eastern Europe and Asia.

The most comprehensive review of embryological development of carp eggs was reported by Verma.^{90,94} Frank⁹⁵ provided a review of egg development which included photographs of each stage. Eggs of carp are approximately 1.9 mm in diameter after water hardening occurs. The unfertilized ovum is centrolecithal, containing yolk material accumulated in the center of the egg surrounded by a cushion of cytoplasmic fluid. The first cleavage begins approximately 30 minutes after fertilization and is meroblastic. The first cleavage divides the blastodisk in half and is followed by divisions which give 4, 8, 16, 32, 64 and 128 cells. Each cell is known as a blastomere; and as cell division

progresses, blastomeres become increasingly difficult to discern and spread to the opposite side of the yolk. This marks the end of cleavage. Blastula follows and is characterized by a hollow ball of cells at one end of the yolk.

The next stage of development is known as gastrulation. During this period, the blastoderm begins to flatten and spread over the yolk, forming the germ ring. Gastrulation is complete when the germ ring has closed. Somites may be visible along the spinal chord at this time. Subsequent development can be divided into a number of stages which are characterized by the formation of organs in the embryo. Collectively, this is known as organogenesis. Several key stages during this developmental period are presented in order of their appearance: increasing number of somites; formation of the optic cup; formation of the heart; motility; circulation; hatching.^{96,97,98}

The length of time it takes an egg to hatch after fertilization depends upon the kind of egg and the environmental conditions, particularly temperature. Higher temperatures impede the developmental process. Carp eggs that are incubated at 24° C hatch in approximately 72 hours. The newly hatched larvae are about 6-7 mm in length and retain a small amount of yolk material.

Figure 6 shows seven stages of development of carp eggs incubating at 24° C. At 30 minutes the blastodisk is beginning to appear at the top of the yolk. At 50 minutes the blastodisk has two cells and at 2 hours, 8 cells. When the embryo is 16 hours old, optic cups are appearing and somites are visible along the notochord. Organogenesis

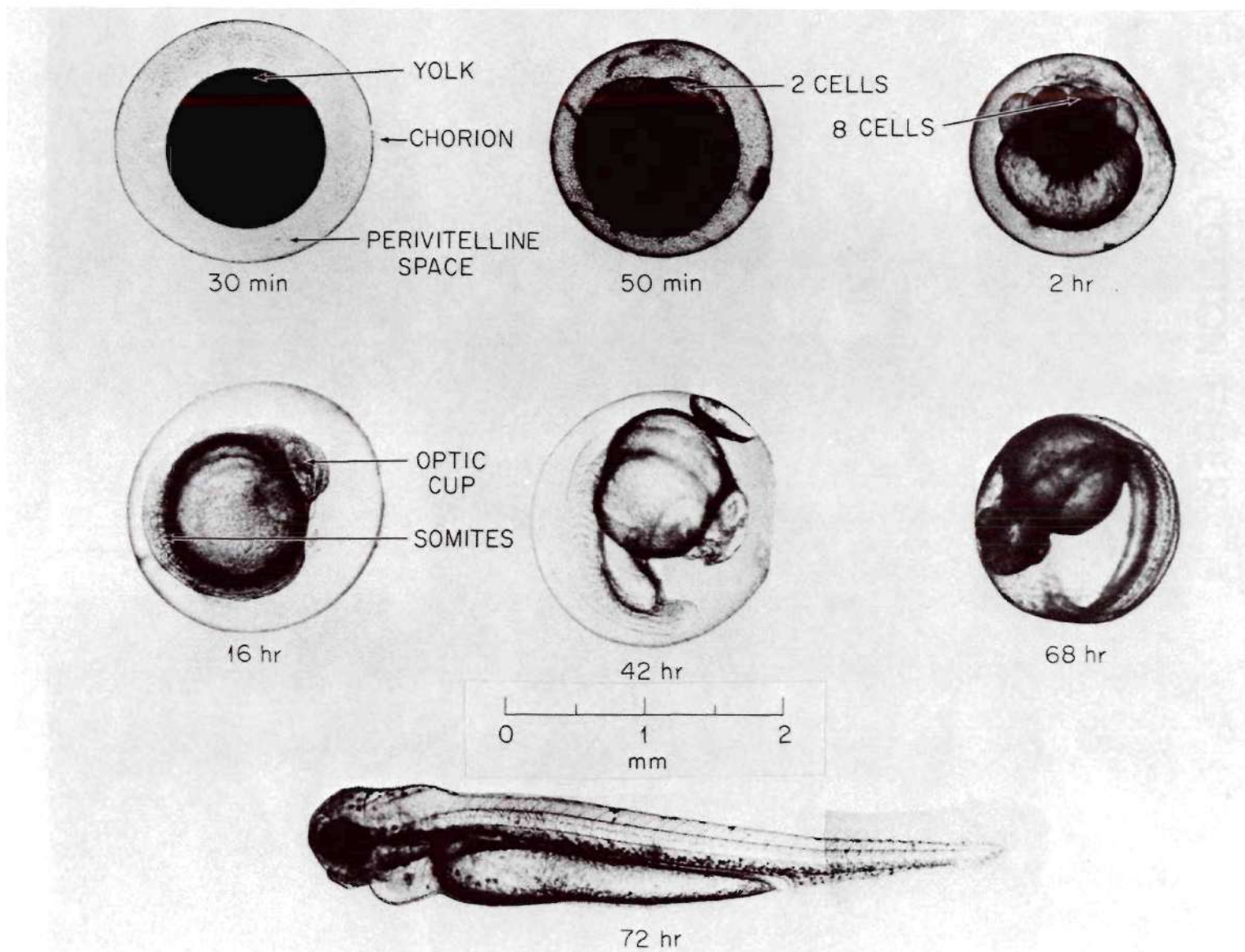


Figure 6. Embryonic Development of Carp Eggs

continues and when the egg is approximately 42 hours old and pigmentation of the eyes and body begins. At 68 hours, the larva is fully developed and ready for hatching.

Cyprinus carpio Eggs as an Aquatic Test Organism. Numerous toxicity test systems would have been suitable for conducting this research. A comprehensive review of some of the test systems as applied to chemical mutagens was published by Hollaender.⁹⁹ There are, however, several distinct advantages to using *C. carpio* eggs for radiological and chemical toxicity tests. Some of these advantages are summarized below:

1. The fecundity of the ripe female carp implies a large sample from which test organisms may be taken.
 2. The short developmental period of *C. carpio* eggs permits collecting test results rapidly.
 3. Approximately 100% fertilization occurs with laboratory spawning and greater than 97% hatch of the eggs can be expected.
 4. Eggs may be exposed to test solutions immediately after fertilization when the embryo is most sensitive to radiological or chemical mutagens.
 5. Carp are found throughout most of the world, having been transplanted extensively outside of their natural range.
- Therefore, the results of these experiments can be related to the exposure of other biota in aquatic ecosystems.

Several disadvantages to *C. carpio* eggs as a toxicity test system are the following:

1. Availability of eggs depends upon the spawning season which lasts approximately six weeks. Experiments must be well organized prior to conducting them.
2. Carp must be obtained from the field. Maintaining a laboratory strain of carp of reproducing age requires large storage pools and expensive materials.
3. The sensitivity of the toxicity test depends upon the type of mutagen being tested. Eggs are relatively insensitive to materials that do not readily penetrate the chorion.
4. Laboratory spawning is not a natural process and may affect the outcome of toxicity tests.

Pimephales promelas (Rafinesque)

Reproductive Biology/Embryology. The most significant disadvantage to using *C. carpio* eggs for this research was the short spawning period. The hazards associated with experimental research on plutonium and uranium require that experiments be conducted in specially designated areas. At the Oak Ridge National Laboratory such space was available although limited. For this reason and because the *C. carpio* spawning season during 1975 was relatively short, an additional toxicity test system was developed which used the eggs of fathead minnows, *Pimephales promelas* (Rafinesque). This test system provided additional data and allowed key experiments to be repeated using another species of fish.

The reproductive biology of *P. promelas* has been reported in the literature although this member of the Cyprinidae family has not been studied as extensively as *C. carpio*.^{74,100,101} Information could not

be found in the literature which explicitly described the manner in which eggs of *P. promelas* are laid and fertilized. Therefore, this process is reviewed in the following paragraphs based upon this author's observations during these experiments.

In natural waters, the male fathead minnow locates a suitable nesting site which is usually on the underside of floating objects or vegetation.¹⁰¹ In laboratory aquaria, artificial nests were constructed of plastic pipe (7.0 cm I.D.) cut in half and approximately 7.6 cm in length. Male fatheads which are ready for spawning continuously guard the nesting site. Other minnows that enter the nest are driven away. During the time that the nest is being prepared, the male fathead continuously brushes the surface of the nest with his head, dorsal fin, and caudal fin. This apparently maintains a clean surface on which the eggs may be attached. While males are guarding nests, their color becomes dark and markings are distinctive. Light grey tubercles protrude from the forehead. The spawning male is also easily identified by two vertical white stripes located ahead of the dorsal fin.

When a female is ready to spawn, she enters the nest. The male and female swim together, the male rubbing the area of the female just behind the pelvic fin with his pelvic fins. Eggs are released and fertilized as the pair flip to one side, then back to the vertical position in a continuous motion. Eggs are attached to the surface by an adhesive material on the chorion. The number of eggs released each time could not be accurately determined; however, it appears that it is most often between one and five. Eggs are grouped together usually in rows

and occasionally are layered as many as three eggs deep. When the female has expended her supply of eggs, she is forced to leave the nest by the male. During the development period, the male continues to rub the dorsal and caudal fin gently against the eggs. This likely prevents damage to the eggs by fungus.

The embryology of *P. promelas* eggs is very similar to the embryology of *C. carpio* eggs although development takes approximately 168 hours at 24° C. Niazi¹⁰¹ has published a detailed review of the embryology; however, it was necessary to produce a series of photographs to determine more precisely the age of freshly fertilized eggs in water at a temperature of 25° C. This "biological clock" is illustrated in Figure 7. Since eggs are most sensitive during the early stages of development they were not used for toxicity tests if they could not be placed in the test solution before blastula was completed.

Although Niazi observed the embryological development of *P. promelas* eggs in detail, he made several attempts without success to obtain specimens for observation by placing spawning males and females in aquaria in the laboratory. Stripping techniques were also unsuccessful for providing fertilized eggs in large quantities and at regular intervals. For this research an abundant supply of freshly fertilized eggs was necessary. This supply was achieved after considerable effort to establish stable laboratory environmental conditions and a suitable technique for retrieving the eggs and using them in experiments without physical injury. This method will be reviewed in detail in the following chapter.

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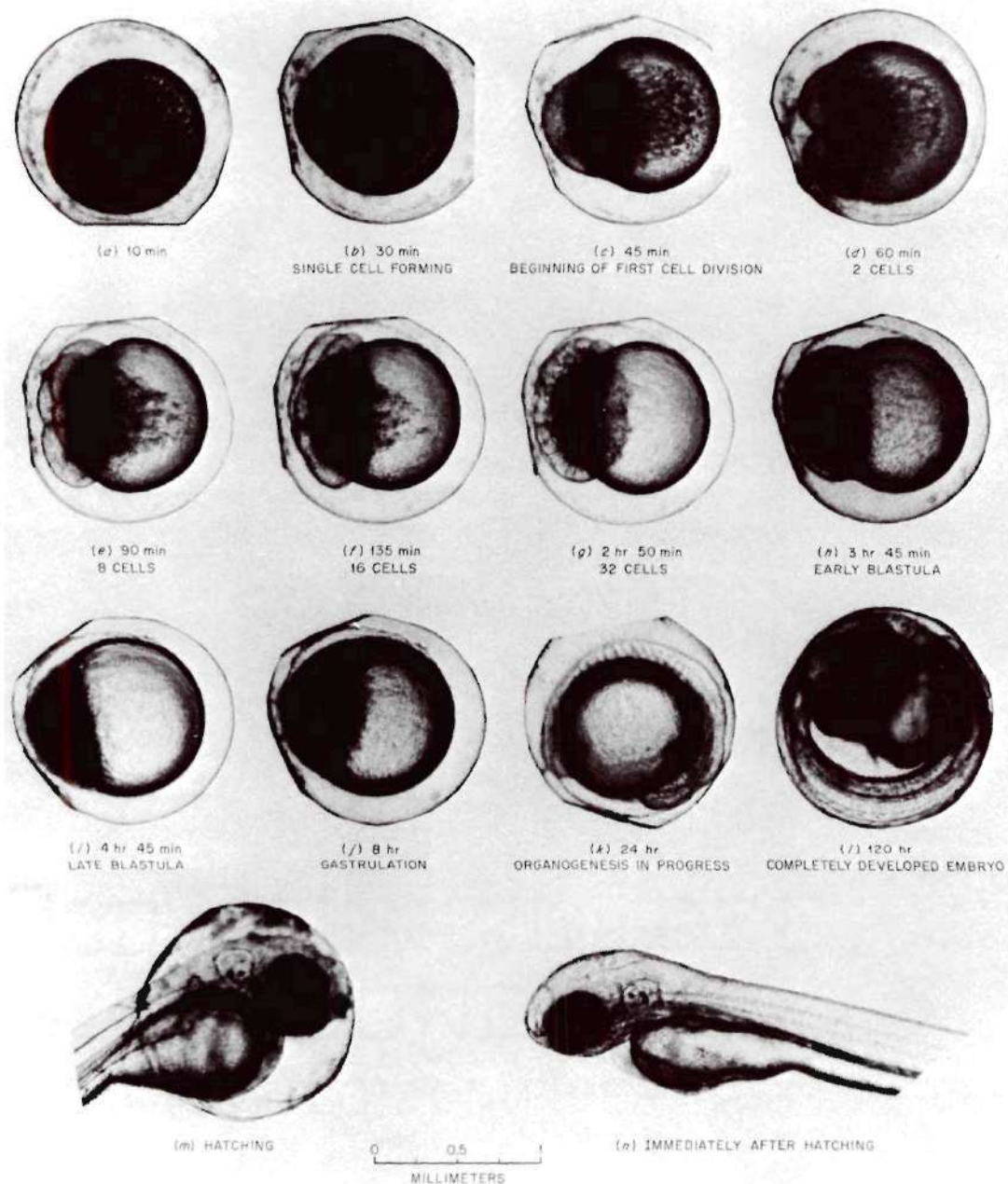


Figure 7. Embryonic Development of Fathead Minnow Eggs

Pimephales promelas Eggs as an Aquatic Test Organism. The advantages to using *P. promelas* eggs for radiological and chemical toxicity tests are summarized below:

1. Eggs can be obtained in the laboratory by normal spawning of males and females throughout the year.
2. The short developmental period of *P. promelas* permits rapid test results.
3. A relatively high percent hatch of eggs can be obtained.
4. The small size of the adult fathead minnow is optimum for conducting laboratory experiments without the need for extensive space or holding facilities.
5. Fathead minnows are found throughout most of the United States, transplanted extensively outside their original range. Therefore, the results of these experiments can be related to the exposure of other biota in aquatic ecosystems.

Several of the disadvantages to using *P. promelas* eggs for radiological and chemical toxicity tests are also listed below.

1. Eggs are usually into the early developmental stages before they can be placed in the test solution.
2. The number of eggs laid at one spawning varies significantly and often may not be sufficiently large for experimental use.
3. The fathead minnow is a nest builder; therefore, development of eggs that are placed in the test solution may be affected by the absence of the male.

4. The small size of *P. promelas* eggs makes quantitative experiments to determine membrane penetration by plutonium and uranium difficult to perform.

Summary

The reproductive biology and embryology of *C. carpio* and *P. promelas* have been reviewed from the literature and observations by this author. There are significant advantages to using eggs of these teleosts as a test organism to determine the toxicity of plutonium and uranium to aquatic biota.

Frequency of Abnormal *C. carpio* and *P. promelas* Larvae Hatched Under Controlled Conditions

In order to evaluate radiation-induced effects in fish eggs, it was important to determine initially baseline data on the statistics of abnormal hatchings occurring in the presence of natural background radiation. Frank⁹⁵ observed four abnormal *C. carpio* larvae among a total of 1,592 larvae that hatched under control conditions. Only embryos with gross malformations such as curvature of the spinal column were recognized as abnormalities. Therefore, less conspicuous teratological effects may have been present such as malformations involving the eyes which were reported by McGregor and Newcombe.⁴⁷

Trabalka⁷⁴ found four basic types of abnormalities among *P. promelas* larvae. The most frequent abnormality was a dorsoventral curvature of the spinal column, usually associated with a severe "hunch-back" deformation. A second spinal abnormality was lateral curvature of the spinal column. Non-spinal teratological effects were abdominal

twins, and incompletely developed caudal fins. Trabalka reported finding seven abnormal larvae among 2,573 specimens collected. Six of the abnormalities involved spinal curvature.

During the present experiment only gross abnormalities involving curvature of the spinal column were observed. This may have been due primarily to the complex nature of conducting the experiments inside a glove box.

Mechanism for Plutonium and Uranium Penetration of the Chorion

As previously mentioned, there are no data in the literature that suggest the extent to which plutonium or uranium in solution penetrate the chorion of a developing fish egg. Only one author has reported values for membrane penetration by specific elements.⁶⁸ Considerable data are available, however, on the diffusion through the chorion of certain elements essential in egg development.

The chorion of *C. carpio* and *P. promelas* eggs is a mechanical structure produced at laying. Eggs usually take on additional water after they are laid and fertilized and then undergo a process of hardening. The chorion is considered to be impermeable to colloidal particles and large molecules. Water continues to pass through the membrane in both directions throughout development. Ions of calcium and sodium are absorbed from the medium during development of fish eggs so that upon hatching, the final concentration of these elements in the eggs is several times greater than the initial concentration.¹⁰² The toughened chorion also permits the passage of oxygen ammonia, hydrogen, and hydroxyl ions into the egg. On the other hand, carbon dioxide, potassium and phosphate are lost to the environment during development.¹⁰³

Because of the complex chemical behavior of both plutonium and uranium, it was difficult to predict the quantity of these materials that would pass through the chorion. Complex ions of these elements are considerably larger than the ions of the inorganic salts discussed above. Also, it cannot be readily assumed that plutonium or uranium would be used in metabolic processes. Therefore, prior to conducting these experiments, it was thought that plutonium and uranium would be absorbed by the egg at approximately the fractions listed by the International Commission on Radiological Protection for absorption through the GI tract of human beings. These fractions are 3×10^{-5} for plutonium and 10^{-2} for uranium.¹⁰⁴

The mechanism by which chorion penetration occurs is not known. However, Ivanov suggested transport was by passive diffusion. He also reported that adsorption of radionuclides on the cell membrane was independent of activity in the embryo and also independent of the type of egg.⁷²

Radionuclides Used in This Study

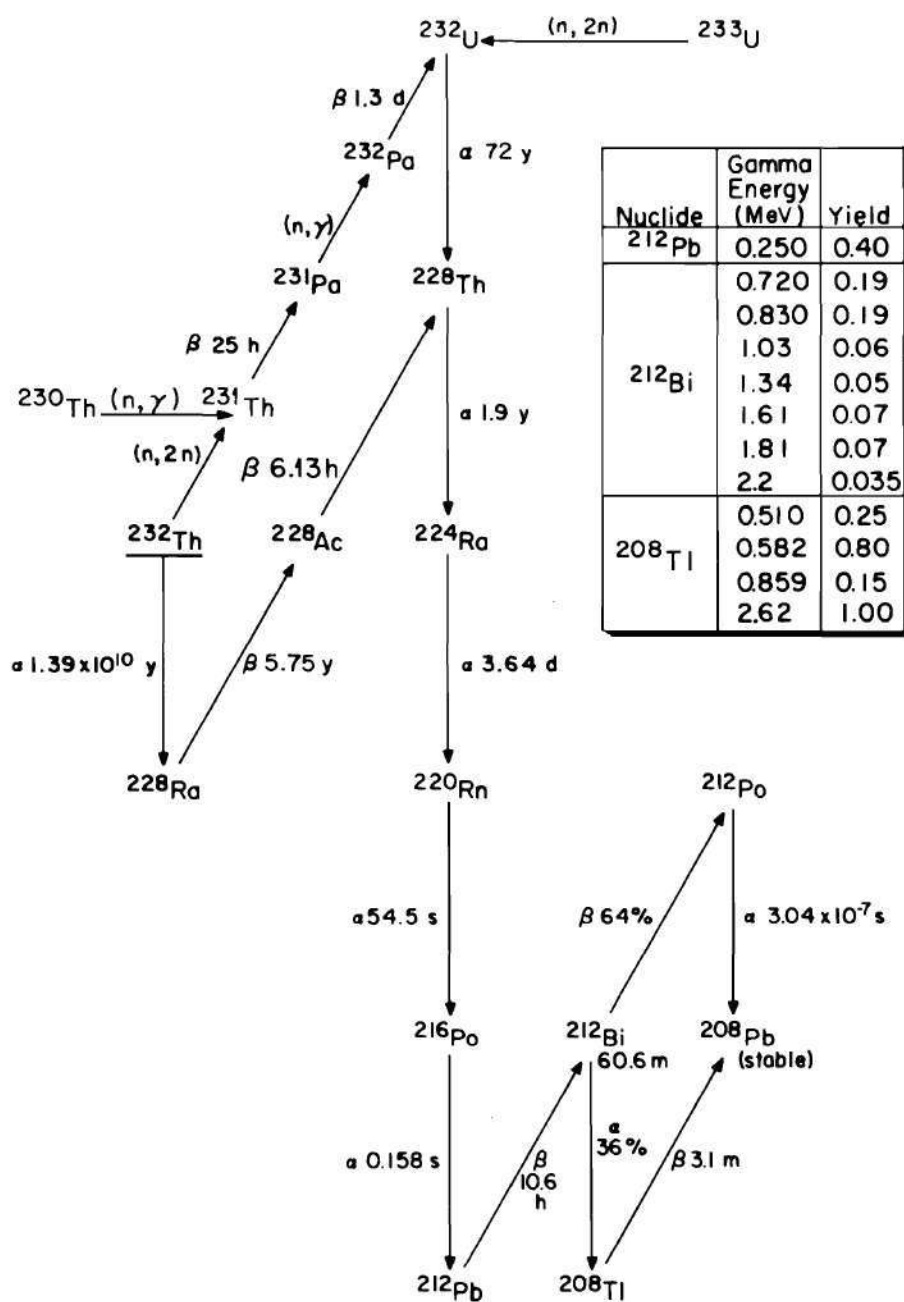
Uranium

Uranium-232. Uranium-232 was selected for use in this study because of its potential significance in the thorium fuel cycle as discussed in Chapter I, and because this isotope of uranium possesses high specific activity relative to other alpha emitting uranium isotopes (21.2 Ci/g). This latter property implies that the effects produced by ^{232}U are radiological rather than chemical.

Uranium-232 is produced in the thorium fuel cycle from neutron interactions with ^{230}Th , ^{232}Th , and ^{233}U (Figure 8). The $^{232}\text{Th} (n, 2n) ^{231}\text{Th}$ fast neutron reaction with a threshold energy cross section of 0.004 b and the $^{233}\text{U} (n, 2n) ^{232}\text{U}$ fast neutron reaction with a threshold energy cross section of 0.002 b are not as significant as the $^{230}\text{Th} (n, \gamma)$ radiative capture reaction.¹⁰⁵ This interaction has a thermal neutron cross section of 23.2 b.¹⁰⁵ Therefore, the content of ^{230}Th in thorium ore affects the concentration of ^{232}U which is ultimately mixed with other uranium isotopes in the fuel.

Natural thorium consists of ^{232}Th ; however, it may contain small amounts of ^{230}Th , a daughter of ^{234}U . Estimates of ^{230}Th in ^{232}Th range between 0.01 ppm in typical thorite-vein ore to 100 ppm, which is an upper limit for raw material thorium for commercial HTGR's.¹⁰⁶ These levels of ^{230}Th result in ^{232}U concentrations at equilibrium recycle of 360 ppm to 1160 ppm.¹⁰⁶

The ^{232}U decay chain is a member of the 4n or thorium series. In this decay chain there is no long-lived "stopping" nuclide such as exists in other actinide decay chains. This property implies that the effective absorbed energy per disintegration, $\Sigma \text{EF(RBE)}_n$, of ^{232}U is high when compared to other radionuclide chains. The moderate half-life of ^{232}U , 72 years, and the fact that there is no "stopping" radionuclide in the chain result in a rapid in-growth of daughters after ^{232}U is initially isolated. Therefore, the build-up of external exposure from penetrating radiation must be considered in the design of any experiment using ^{232}U .

Figure 8. Production and Decay of ^{232}U and ^{228}Th

The ^{232}U for these experiments was obtained from the Oak Ridge National Laboratory. The isotope was originally produced at ORNL in 1962 by irradiations of ^{231}Pa borrowed from Great Britain.¹⁰⁷ The mass spectrum of the original batch and the mass spectrum calculated for 1975 are listed below.

	1962	1975
<u>Isotope</u>	<u>Atom %</u>	<u>Atom %</u>
^{232}U	99.2430	99.1450
^{233}U	0.7209	0.8162
^{234}U	0.0083	0.0094
^{235}U	0.0014	0.0016
^{236}U	0.0013	0.0015
^{238}U	0.0236	0.0294

The original batch of ^{232}U had not been used for several years before this experiment. Therefore it was necessary to chemically separate the ^{232}U from its daughters. This provided a radiochemically pure sample of ^{232}U . Five milligrams of ^{232}U as $\text{UO}_2(\text{NO}_3)_2$ in a volume of two milliliters were received on April 21, 1975. The unshielded exposure rate on the bottom of the vial was 460 mR/hour. This basic stock solution was shielded in a lead pig. Because this radionuclide is considered to be of very high radiotoxicity (class 1) according to the Oak Ridge National Laboratory Health Physics Manual,¹⁰⁸ all experiments involving ^{232}U had to be conducted in specially designated alpha isolation facilities.

Uranium-233. Uranium-233 was selected as a tracer to determine the penetration of uranium through the chorion and the distribution of uranium in the egg contents. This isotope of uranium is considered to have moderate radiotoxicity rather than very high radiotoxicity characterized

by ^{232}U . A good chemical laboratory with a hood equipped with high efficiency filters is adequate for experiments with ^{233}U .¹⁰⁸

A sample of 240 mg of ^{233}U as U_3O_8 was received on February 19, 1975 for use in this experiment. Mass spectrum analysis determined that it consisted of the following isotopes.

Isotope	Atom %
^{232}U	0.0008
^{233}U	99.5400
^{234}U	0.1840
^{235}U	0.0620
^{236}U	0.0130
^{238}U	0.2030

The original sample, U_3O_8 , was converted to $\text{UO}_2(\text{NO}_3)_2$ and adjusted to a volume of two milliliters.

Uranium-233, an alpha emitter, is a member of the neptunium or $4n + 1$ series and, like the other members of this series, must be artificially produced. This sample of high purity ^{233}U was produced from fertile ^{232}Th through neutron capture and successive beta decays of ^{233}Th and ^{233}Pa .

Uranium-235, 238. In order to determine the chemical toxicity of uranium to developing embryos, ^{235}U and ^{238}U were selected as test radionuclides. The low specific activity of these isotopes implies that any observed effects to biota which had been exposed to ^{235}U and ^{238}U are due to chemical toxicity. Therefore by comparing the effects from ^{232}U to ^{235}U or ^{238}U , one can determine the difference between chemical toxicity and radiological toxicity.

The isotopic composition of the ^{235}U and ^{238}U used in this experiment as determined by mass spectrum analysis is listed below.

<u>^{235}U</u>		<u>^{238}U</u>	
<u>Isotope</u>	<u>Atom %</u>	<u>Isotope</u>	<u>Atom %</u>
^{233}U	<0.0001	^{233}U	<0.0001
^{234}U	0.0290	^{234}U	<0.0001
^{235}U	99.9120	^{235}U	0.0011
^{236}U	0.0160	^{236}U	<0.0001
^{238}U	0.0414	^{238}U	99.9990

Approximately 200 mg of each isotope as U_3O_8 was received on February 19, 1975. This was converted to $\text{UO}_2(\text{NO}_3)_2$ and adjusted to a volume of two milliliters. The Oak Ridge National Laboratory Health Physics Manual lists both ^{235}U and ^{238}U as class 4 isotopes having slight radiotoxicity;¹⁰⁸ a good chemical laboratory is adequate for experiments involving ^{235}U or ^{238}U .

Both ^{235}U and ^{238}U decay by alpha emission and are naturally occurring radionuclides. The high enrichment for these samples was obtained by electromagnetic mass separation.

Plutonium

Plutonium-238. Radiochemically pure ^{238}Pu possesses ideal physical properties to determine the effects from plutonium radioactivity to developing fish embryos. This isotope of plutonium has relatively high specific activity, 17.1 Ci/g, and may be obtained at reasonable cost and high isotopic purity. The specific activity of ^{238}Pu is very similar to the specific activity of ^{232}U . This property permits a comparison of the radiological effects of the two elements without a significant difference between the masses involved.

Plutonium-238 is a member of the $4n + 2$ or uranium decay chain. The decay of ^{238}Pu is "blocked" after alpha emission by the first daughter, ^{234}U , which has a long half-life, 2.47×10^5 years (Figure 9a). Five milligrams of ^{238}Pu as PuO_2 were initially obtained for these experiments and converted to $\text{Pu}(\text{NO}_3)_4$. The isotopic composition of this sample is listed below.

Isotope	Atom %
^{238}Pu	97.380
^{239}Pu	1.670
^{240}Pu	0.887
^{241}Pu	<0.040
^{242}Pu	0.022
^{244}Pu	<0.001

The Oak Ridge National Laboratory Health Physics Manual lists ^{238}Pu as a class 1 radionuclide having very high radiotoxicity.¹⁰⁸ Therefore experiments with ^{238}Pu must be conducted in specially designed alpha isolation facilities.

Plutonium-244. The chemical toxicity of plutonium has not been demonstrated because very long-lived isotopes of this element are rare and extremely difficult to produce. Only one plutonium isotope, ^{244}Pu , has a sufficiently long half-life, 8.3×10^7 years, which is needed to conclusively determine the chemical toxicity of plutonium.

A relatively pure sample of ^{244}Pu was acquired for this experiment in order to investigate the chemical toxicity of plutonium to aquatic biota. This material was originally a by-product from the production of approximately five kilograms of ^{244}Cm from ^{239}Pu .¹⁰⁹ For the most efficient production, the conversion of ^{239}Pu to ^{244}Cm was made

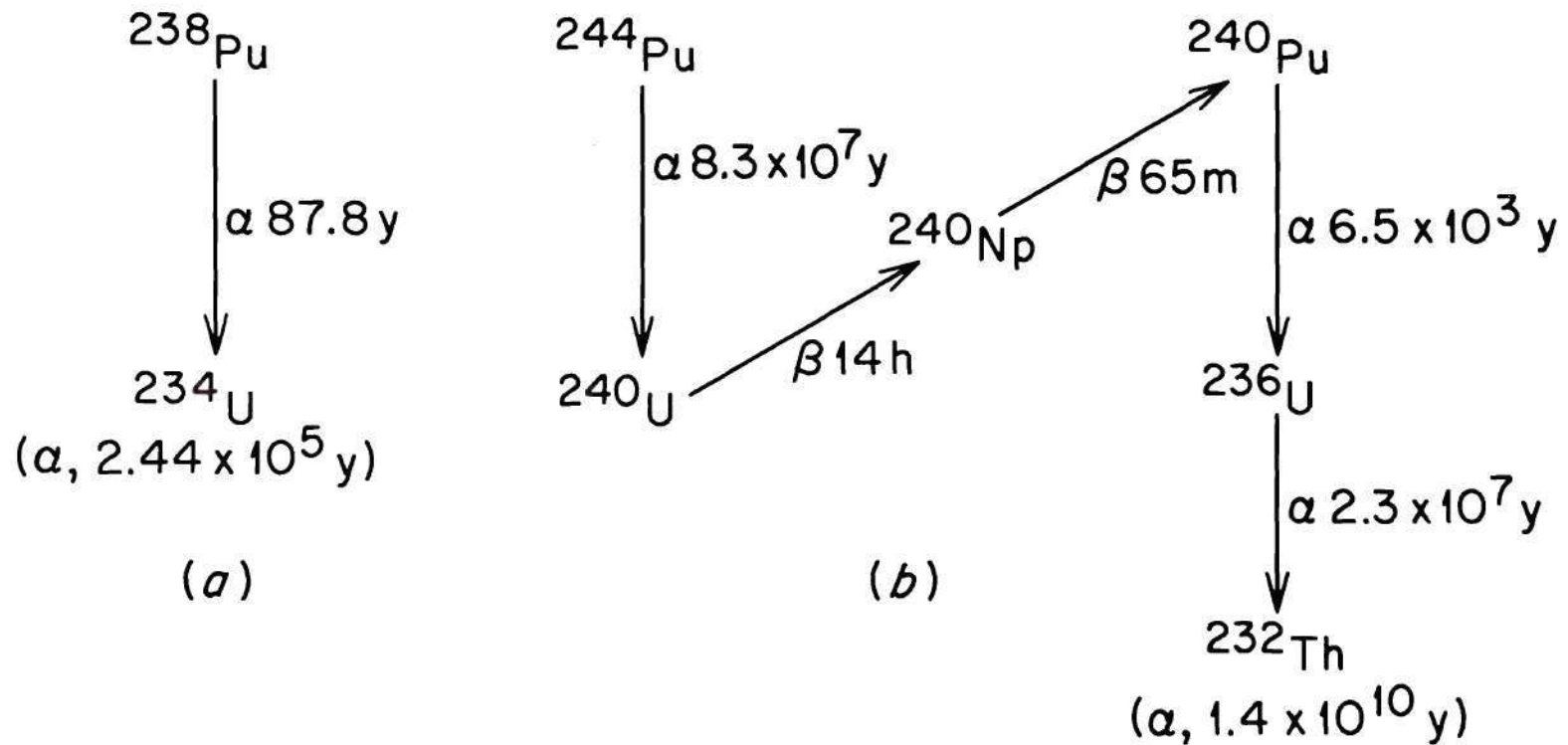


Figure 9. Decay of ^{238}Pu and ^{244}Pu

in several stages. First, ^{239}Pu was enriched to ^{240}Pu . Second, ^{242}Pu was produced by successive neutron captures. Third, the ^{242}Pu was fabricated into targets and placed in the outer housing high flux region of a reactor at the Savannah River Plant. The resulting plutonium, americium, and curium were separated chemically. The plutonium recovered had a ^{244}Pu content of approximately 25%. This sample was further enriched to more than 98% (atom) by electromagnetic mass separation (calutron).^{110,111}

Isotopic composition of the ^{244}Pu is listed below with the corresponding radioactivity for 1 mg of sample.

Isotope	Atom %	Activity in 1 mg of Sample	
		(μ Ci)	
^{238}Pu	0.003	1.7×10^{-1}	(α)
^{239}Pu	0.001	1.8×10^{-2}	(α)
^{240}Pu	0.305	7.0×10^{-1}	(α)
^{241}Pu	0.074	7.3×10^1	(β ⁻)
^{242}Pu	1.050	4.0×10^{-2}	(α)
^{244}Pu	98.570	1.7×10^{-2}	(α)
Total		0.95 μCi/mg (α)	

Plutonium-244 is a member of the 4n or thorium series. The radioactive decay is illustrated in Figure 9b. Approximately 65 mg of ^{244}Pu initially received as PuO_2 were used in these experiments. The chemical form was converted to $\text{Pu}(\text{NO}_3)_4$ by dissolving the solid in concentrated nitric acid. The total radioactivity associated with one mg of the ^{244}Pu sample is still relatively high due to the presence of high specific activity impurity isotopes. Most of the alpha radioactivity associated with the sample results from ^{240}Pu (74%) and ^{238}Pu

(18%). Plutonium-244 contributes less than 2% of the total alpha activity. The specific activity of the ^{244}Pu sample used in these experiments (9.5×10^{-4} Ci/g) is a factor of 64 times lower than the specific activity of fissile ^{239}Pu (6.13×10^{-2} Ci/g), the most abundant and important plutonium isotope in the nuclear fuel cycle.

Table 7 summarizes some physical and chemical properties of the uranium and plutonium isotopes as well as their protection criteria.

Chemical Form of Plutonium and Uranium for This Study

The most common chemical forms of uranium and plutonium found in the literature on biological studies include oxides, nitrates, fluorides, chlorides, and citrates.² It was necessary to consider the following physical and chemical properties in order to select the best chemical form for this study.

1. Valence state and chemical form of plutonium and uranium in natural ecosystems.
2. Hydronium ion concentration similar to environmental waters.
3. Highest solubility possible for both elements.
4. Toxicity of the anion.
5. Chemical form that is compatible for both elements.

Although the chemistry of plutonium in laboratory concentrations has been well documented in the literature,^{6,114,115,116} the most likely chemical form and valence states of plutonium in the environment have not been well established. The most likely forms of release to the environment are PuO_2 , mixed Pu-U oxide, silicates, and liquids of various types. Airborne particles depositing near their sources would be expected

Table 7. Physical and Chemical Properties and Protection Criteria for Isotopes Used in This Study

Isotope	Half-Life ¹¹² (Years)	Specific Activity (Ci/g)	168 Hour/Week (MPC) _a (Soluble) ^{104,113} (μ Ci/cc)	Toxicity Class ¹⁰⁸	Oxidation States Found In Aqueous Solutions ²
²³² U	7.20×10^1	2.12×10^1	3×10^{-11}	Very High	U ³⁺ , U ⁴⁺ , UO ₂ ⁺ , UO ₂ ²⁺
²³³ U	1.62×10^5	9.72×10^{-3}	2×10^{-10}	Moderate	U ³⁺ , U ⁴⁺ , UO ₂ ⁺ , UO ₂ ²⁺
²³⁵ U	7.04×10^8	2.14×10^{-6}	2×10^{-10}	Slight	U ³⁺ , U ⁴⁺ , UO ₂ ⁺ , UO ₂ ²⁺
²³⁸ U	4.47×10^9	3.33×10^{-7}	3×10^{-11}	Slight	U ³⁺ , U ⁴⁺ , UO ₂ ⁺ , UO ₂ ²⁺
²³⁸ Pu	8.78×10^1	1.71×10^1	6×10^{-13}	Very High	Pu ³⁺ , Pu ⁴⁺ , PuO ₂ ²⁺ , PuO ₂ ²⁺ , PuO ₅ ³⁺
²⁴⁴ Pu	8.30×10^7	1.76×10^{-5}	6×10^{-13}	a	Pu ³⁺ , Pu ⁴⁺ , PuO ₂ ⁺ , PuO ₂ ²⁺ , PuO ₅ ³⁺

^aPlutonium-244 was not listed in the Oak Ridge National Laboratory Health Physics Manual; however, because of the presence of impurity plutonium isotopes, it was assumed that ²⁴⁴Pu had very high radiotoxicity.

to be coarse and relatively inert, while those depositing remotely would be more reactive.¹¹⁷ The major difficulty in studying environmental levels of plutonium is its very low concentration in terrestrial and aquatic media. Typical concentration in ocean water and fresh water is approximately 10^{-12} ppm.¹¹⁸

The chemical form and valence state of plutonium in natural waters has been studied by Bondietti et al.¹¹⁹, Silver^{120,121}, and Rozzell and Andelman.¹²² Although conclusive determinations of valence state have not been reported, it is generally accepted that, because of intense hydrolysis and strong complexing tendency, plutonium (IV) should dominate in natural water systems.

Plutonium (IV) forms exceptionally strong complexes with the citrate ion.¹¹⁴ Plutonium citrate has good solubility in water and the pH at which hydrolysis occurs is greater than pH = 8.¹²³ Therefore experiments with Pu(IV) - citrate at pH \approx 7.0 may be performed without hydrolysis. If plutonium remains in solution as a citrate complex, the greatest toxic effect should be observed because the soluble form is more likely to pass through the egg chorion.

The toxicity of the citrate complexing anion to developing embryos has not been reported in the literature. This is discussed later in this thesis.

The colloidal properties of Pu(IV) - citrate in dilute aqueous solutions have been examined by Lindenbaum and Westfall.¹²⁴ Their study was conducted to clarify the conflicting reports in the literature on tissue distribution of plutonium when the same chemical form was being

tested. A common method was established for preparing ultrafilterable plutonium citrate that could be adjusted to a neutral pH for use in biological experiments.

The chemistry of uranium has been the subject of extensive research for many years and there are many comprehensive reviews on uranium chemistry.^{125,126,127,128} Three isotopes of uranium, ^{234}U , ^{235}U , and ^{238}U , are found in nature. In natural waters, the chemical properties of uranium favor the U (VI) valence state, and UO_2^{2+} complexes with carbonate and sulfate are predominant.¹²⁹ Uranium is present at a concentration of approximately 3×10^{-3} ppm in ocean water and 1×10^{-3} ppm in fresh water.¹¹⁸

The uranyl ion forms strong complexes with the citrate ion. Uranyl citrate is soluble in water at neutral pH and most of the UO_2^{2+} remains complexed, provided excess citrate is present in solution.¹³⁰ As with plutonium, the highest toxicity from uranium to fish eggs should be observed when the uranium remains in solution. This physical form assures maximum transport through the membrane.

In view of the above findings, the chemical form and valence state of plutonium and uranium selected for this study were the following:

<u>Element</u>	<u>Valence State</u>	<u>pH</u>	<u>Chemical Form</u>
Plutonium	IV	7.5	Pu(IV) - Citrate
Uranium	VI	7.5	U(VI) O_2 - Citrate

CHAPTER III

MATERIALS AND METHODS

Experimental Facilities

All experimental work for this research was conducted at the Oak Ridge National Laboratory, Oak Ridge, Tennessee, and employed facilities and materials of the Environmental Sciences Division.

Radionuclides Having Very High Radiotoxicity

Among the radionuclides used in this study, ^{238}Pu and ^{232}U are considered to have very high radiotoxicity.¹⁰⁸ Experiments with isotopes in this toxicity class must be carried out in a "Type A" high-level laboratory consisting of glove boxes or hot cell facilities specifically constructed for handling such materials. The use of ^{232}U in an unshielded area is permitted, provided significant daughter build-up has not occurred and penetrating radiation dose rates are not excessive.

Limited space was available in glove boxes operated by the Environmental Sciences Division in the Alpha Isolation Facility, Building 3508, at ORNL. Portions of two glove boxes, one standard "six foot" glove box and one standard "three foot," were available. Basic stock solutions were prepared and maintained in the small glove box. Toxicity and penetration tests were conducted in approximately two-thirds of the large glove box.

Operational procedures for Building 3508 are outlined in ORNL/TM-3938.¹³¹ This manual describes protective clothing, personal monitoring, limited access areas, and bag-in/bag-out techniques required for handling hazardous radioactive materials. A thorough problem safety summary must be submitted and approved before experiments may be conducted in Building 3508. Only personnel who have been trained in proper glove box techniques are permitted to perform experiments in Building 3508. This author began glove box training on February 10, 1975 and continued this training through April. On May 1, 1975 completion of the glove box training was certified (Appendix A). Although two persons must be present for bag-in/bag-out operations, certification permitted the author to continue experiments on weekends and weeknights without assistance from supervisory personnel. Materials can be put into the box through a sphincter port without a complete bag-in procedure. Figure 10 shows the large glove box and sphincter port.

Experiments with Pu were also carried out in a glove box in Building 3508 because of the presence of very high toxicity impurity isotopes. It was also desirable to conduct as many tests as possible with isotopes other than ^{232}U , ^{238}Pu , and ^{244}Pu in the glove box to minimize differences in environmental conditions between experiments.

Radionuclides Having Other Than Very High Radiotoxicity

Building 2001 was the location of experiments involving the less radiotoxic isotopes, ^{233}U , ^{235}U , and ^{238}U . The work area was protected from routine pedestrian traffic and marked with contamination

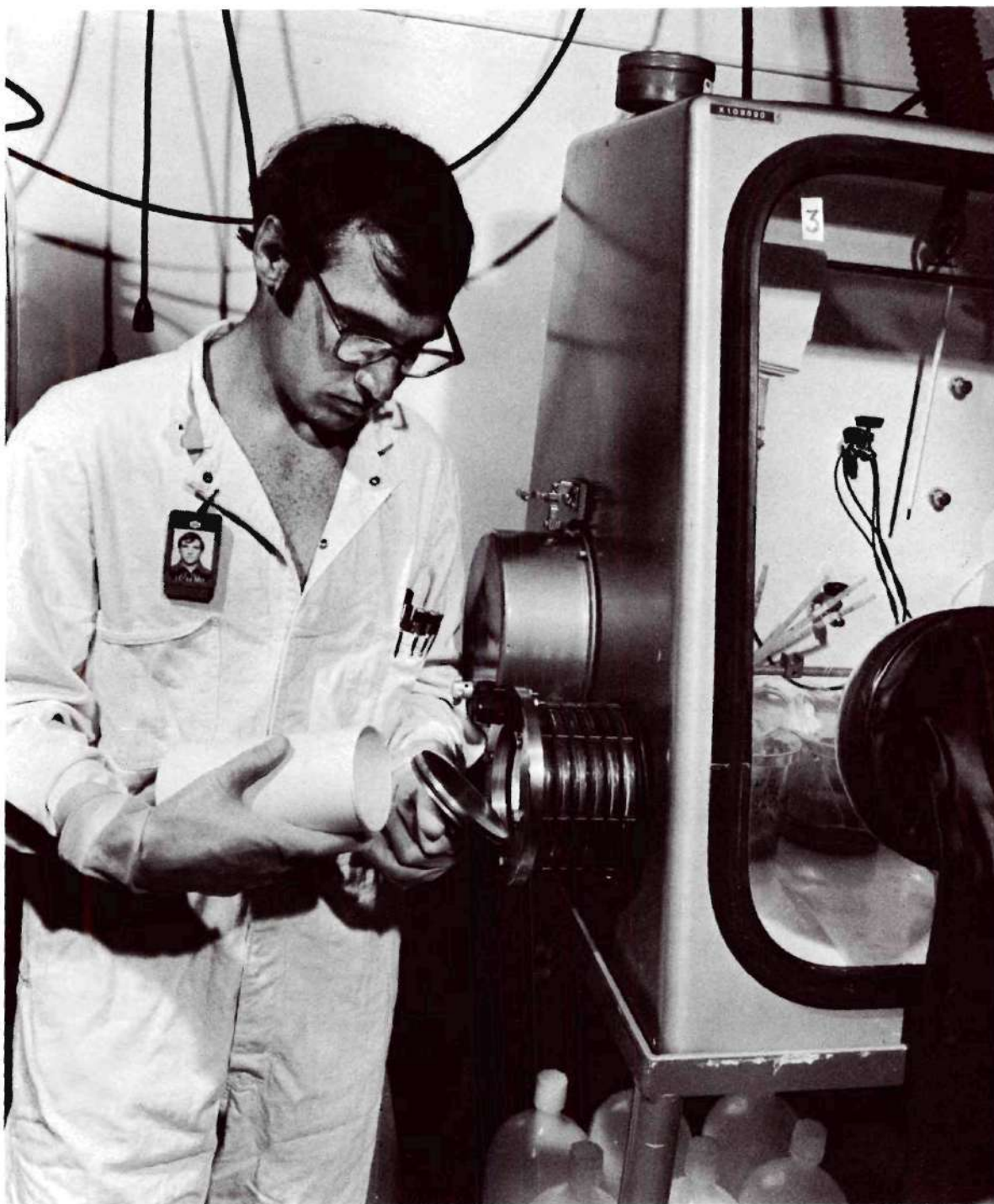


Figure 10. Glove Box and Sphincter Port

zone signs and tape (Figure 11). Experiments with nonradioactive substances were conducted outside of the contamination zone area.

Larvae Survival Studies

Larvae survival was observed by rearing young fry in pans that were placed in environmental chambers in Building 2001. Pans were sampled periodically for alpha radioactivity and special caution was taken to prevent contamination of the environmental chambers or surrounding work areas.

Preparation of Test Solutions

Basic Stock Solutions

Identical plutonium and uranium citrate test solutions were prepared according to the method outlined by Lindenbaum and Westfall.¹²⁴ One milliliter of $^{238}\text{Pu}(\text{NO}_3)_4$ or $^{232}\text{UO}_2(\text{NO}_3)_2$ solution containing approximately 2.5 mg/ml was added to five milliliters of 2% trisodium citrate aqueous solution. Neutralization was accomplished by dropwise addition of concentrated 10 M NaOH before final adjustment to pH 7.5 with 1 M NaOH. Neutralization was continuously monitored with a Corning General Purpose pH Meter having a combination probe and reference electrode to prevent exceeding the desired pH. Approximately 100% of the UO_2^{2+} and the Pu^{4+} are complexed with citrate at a pH = 7.5.^{124,132} Water was then added to the basic stock solution to provide convenient mass and activity concentrations.

Stock solutions of $^{244}\text{Pu}(\text{NO}_3)_4$ and $^{233,235,238}\text{UO}_2(\text{NO}_3)_2$ were prepared similarly except that approximately 15 ml of citrate solution

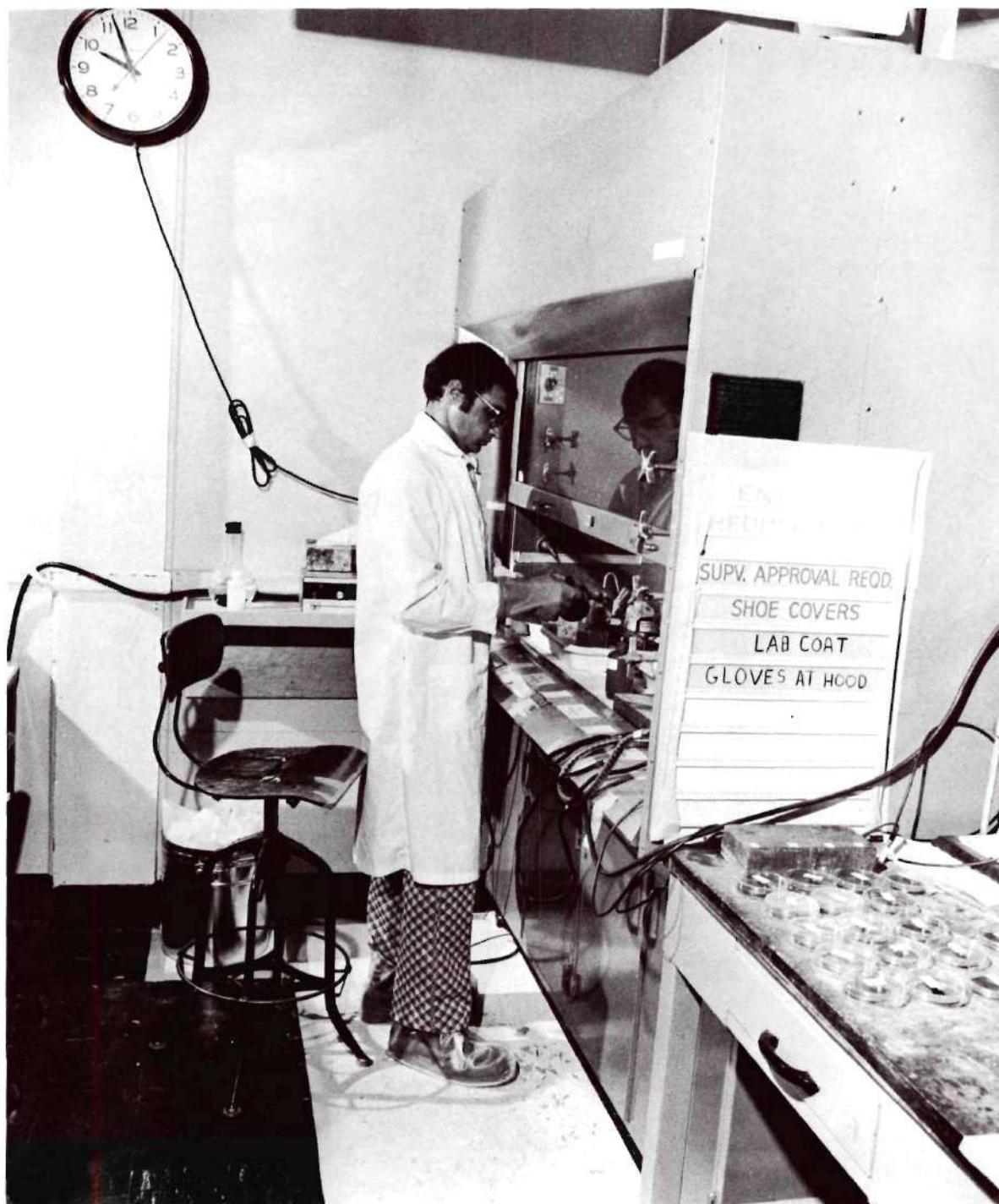


Figure 11. Building 2001 Work Area

were used to assure that excess citrate ion would be present. In each case, no observable precipitate was present in the final stock solution.

Aliquots from basic stock solutions were added to one liter polyethylene bottles containing spring water that had been filtered using 0.45 μ filter paper. In Building 3508, liter bottles containing the radioactivity or mass concentration to be studied were prepared in the small glove box and transferred to the large glove box for use in experiments.

Radioassay of Test Solutions

Each concentration of uranium and plutonium used in this study was analyzed by radioassay after the solution was prepared. Gross alpha analysis was performed by evaporating samples onto five centimeter stainless-steel planchets, heating the planchets to fix the radionuclide to the planchet, and covering the planchets with ZnS wafers. A scintillation detector having an overall efficiency of 49% was used to count alpha activity.

Because ^{232}U has a chain of relatively short-lived daughters and because there is no long-lived stopping radionuclide in the chain, daughter buildup increases the alpha activity present in solution as well as the external dose rate. One objective of this study was to compare the toxicity of ^{238}Pu to ^{232}U . This comparison was based on equivalent concentrations of total alpha activity present in solution rather than individual activity from the ^{238}Pu or ^{232}U . Plutonium-238 daughters did not contribute significantly to the total activity from this isotope because decay is essentially blocked by the first daughter,

^{234}U . Buildup of ^{232}U daughters, on the other hand, caused the fraction of total activity due to ^{232}U itself to decrease during the course of the experiment. Figure 12 shows the fraction of total alpha activity contributed by ^{232}U and alpha emitting daughters as a function of the time elapsed between initial separation and use. Within 30 days after ion exchange separation, the ^{232}U daughters contributed more than 10% of the total alpha activity. By the time the experiments were completed at approximately 200 days, the ^{232}U daughters contributed 46% of the total activity. The mass of ^{232}U present at 200 days was still 99.5% of the original quantity. Gross alpha analysis provided an accurate estimate of the amount of alpha activity present in the test solution at a given time. However, the effective energy per disintegration increases considerably as daughter buildup continues. This increase in effective energy per disintegration was incorporated into dose calculations and will be discussed in further detail in Chapter IV.

The ^{232}U daughters were initially removed from the sample by ion exchange separation on April 18, 1975. Portions of this same stock were used in experiments over the following 180 days. Samples at two intervals from this stock were assayed for ^{232}U and ^{208}Tl by spectrum analysis to verify the content of ^{232}U and daughters in solution. The ratio of ^{232}U and ^{208}Tl activity to total alpha activity for these analyses is listed in Table 8. These data were used to confirm the buildup of daughters in solution and to determine the quantity of ^{232}U present. Ratios computed by spectrum analysis are in good agreement with theoretical values.

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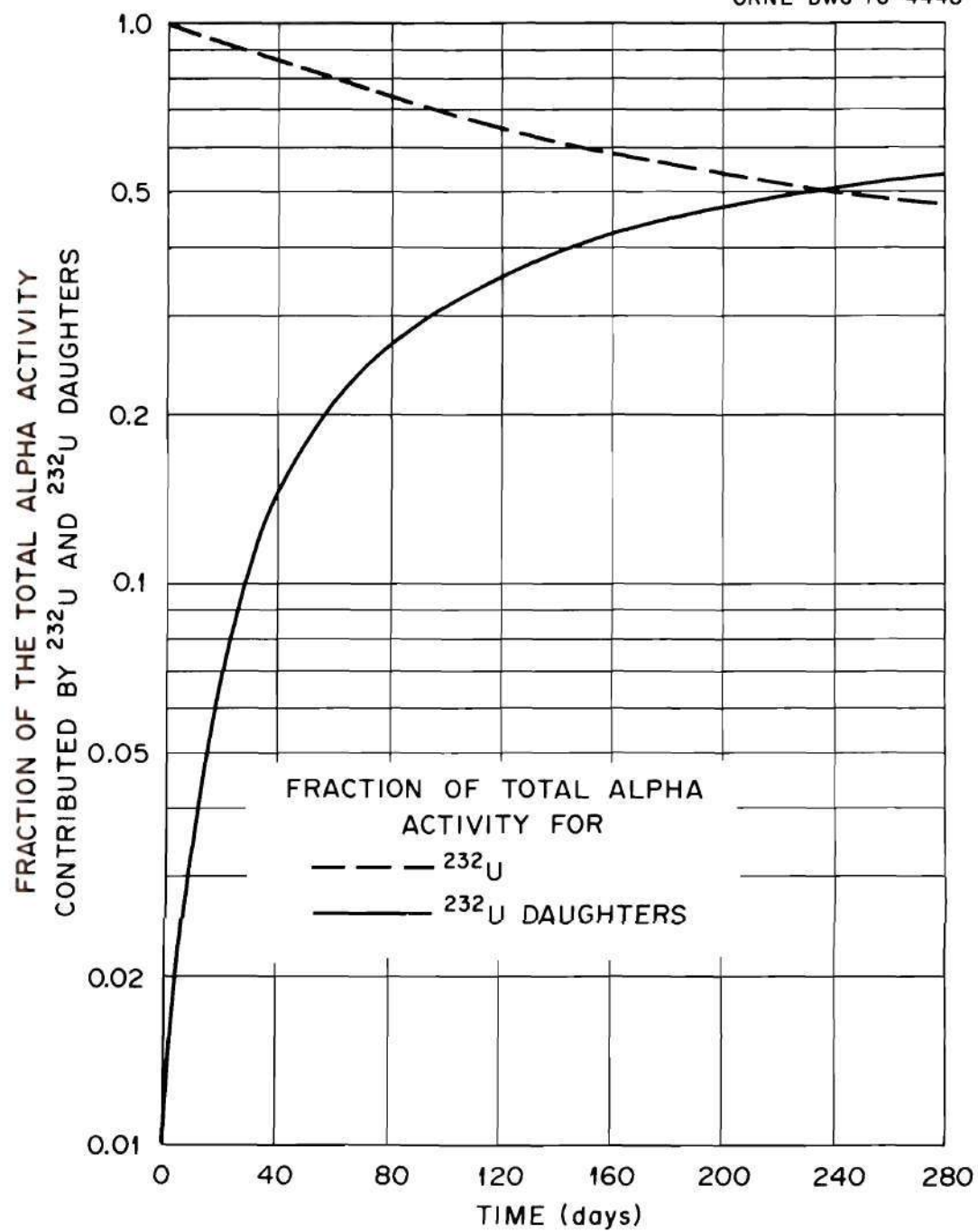


Figure 12. Fraction of the Total Alpha Radioactivity from ^{232}U and ^{232}U Daughters

Table 8. Ratio of ^{232}U and ^{208}Tl Activity to Total Alpha Activity
In A Solution of ^{232}U and Daughters

Date	$\frac{\text{DPM } ^{232}\text{U } \alpha}{\text{DPM Total } \alpha}$	$\frac{\text{DPM } ^{232}\text{U } \alpha}{\text{DPM Total } \alpha}$	$\frac{\text{DPM } ^{208}\text{Tl } \gamma}{\text{DPM Total } \alpha}$	$\frac{\text{DPM } ^{208}\text{Tl } \gamma}{\text{DPM Total } \alpha}$
	(Spectrum analysis)	(Theoretical) ^a	(Spectrum analysis)	(Theoretical) ^a
May 13, 1976	0.944	0.900	0.003	0.006
November 5, 1976	0.650	0.540	0.013	0.012

^aCalculated assuming 100% removal of all ^{232}U daughters on April 18, 1975.

Laboratory Spawning of *Cyprinus carpio*

Collection of Ripe Males and Females

The high toxicity of the radionuclides being investigated, the limited space available to perform the experiments, and the short spawning season of carp required considerable organization and advance planning be given to this study. In order to catch ripe male and female carp early in the spawning season, local residents and other laboratory employees who conduct research in the field were asked to inform the author when they first sighted spawning carp. In addition, known spawning grounds were checked for spawning activity and water temperature on warm days after April 1, 1975.

Figure 13 marks the locations where spawning carp were observed or collected during the spring of 1975. The most active spawning area was on Fort Loudon Reservoir approximately 10 miles west of Knoxville, Tennessee. This is a shallow embayment approximately three acres in size with a heavy outgrowth of willow trees at one end. Since this location was on private property, permission was obtained from the land owner to gather ripe specimens.

Samples were usually collected with a dip net; however, in certain inlets having a narrow channel leading to open water, a seine net was employed. Ripe specimens were checked to determine whether the eggs or sperm had been spent. Those selected for use were carefully placed in 0.44 m (ID) x 0.56 m plastic drums filled with fresh lake water. Males and females were separated during transport. Portable

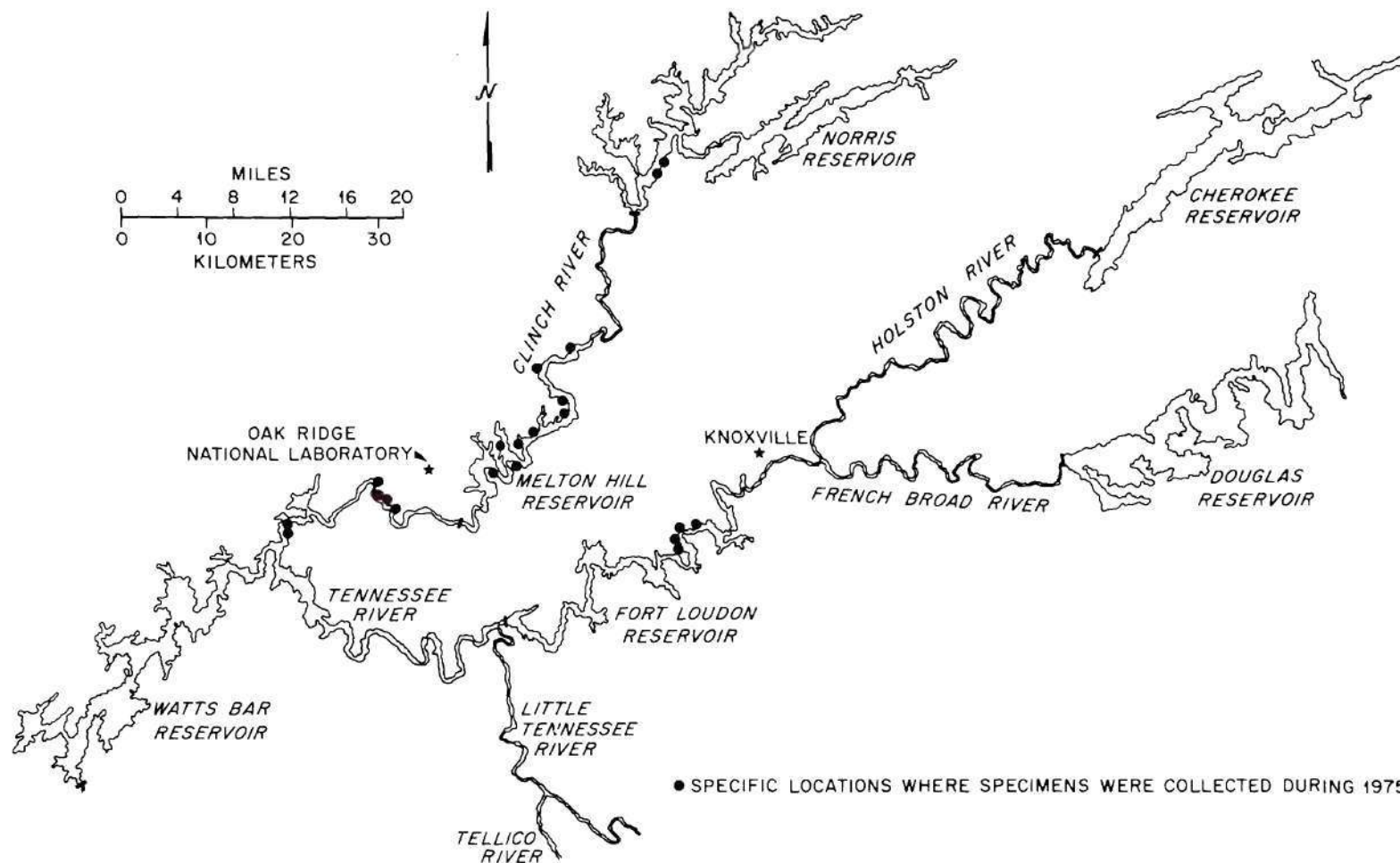


Figure 13. Significant Reservoirs in Proximity to the Oak Ridge National Laboratory Where Active Spawning of *Cyprinus carpio* Occurs During Spring

aerators provided a forced air supply to maintain oxygen concentrations inside the drum.

Figures 14 and 15 illustrate a typical habitat for ripe carp as well as the methods used to collect specimens for this study.

Spawning Procedure

A technique for spawning *C. carpio* in the laboratory was reported in the literature by Blaylock and Griffith.⁸³ Approximately 1,000 eggs were stripped from the ripe female into a Nalgene (trademark) beaker containing 250 ml of 0.6% NaCl in filtered spring water at 25°C. The use of Nalgene provided a significant improvement over Blaylock and Griffith's technique because the eggs did not stick to the beaker walls. The dilute salt solution removes the adhesive coating on the outside of the eggs and prevents them from clumping together. Eggs were swirled vigorously while several drops of milt were stripped from the ripe male into the beaker. Fertilization was almost instantaneous. Eggs were then poured into 3.5 x 8.5 cm (ID) plastic dishes which contained approximately 75 ml of test solution. Eggs still possessed adhesive properties and became fixed to the bottom of the dishes.

The spawning procedure is illustrated in Figure 16. Three persons were needed to carry out the laboratory spawning. Two individuals held a male or female fish while the third held the beaker and poured eggs into the dishes. The optimum number of eggs for one dish is approximately 150. With practice, this number of eggs can be poured and distributed uniformly.



Figure 14. Collection of Ripe Carp in the Field



Figure 15. Collection of Ripe Carp in the Field

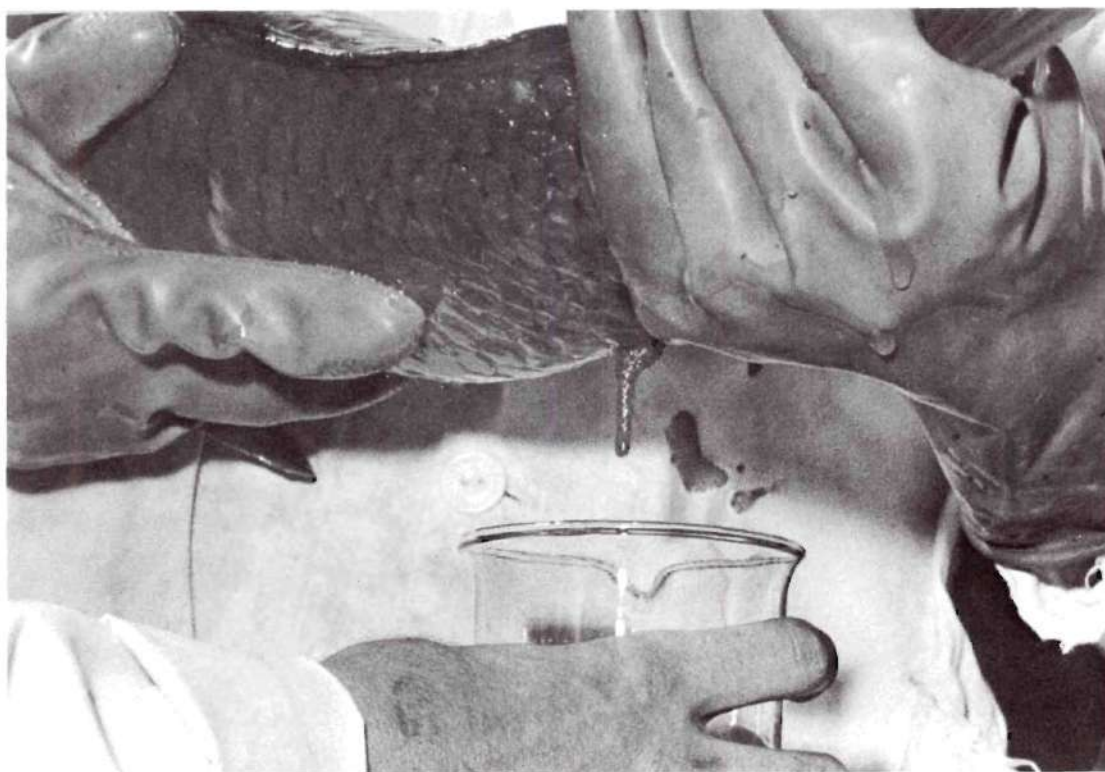


Figure 16. Laboratory Spawning of Carp

The fertilized eggs were not moved or disturbed during the first 30 minutes after fertilization to prevent physical damage during the early development stages. After this period the NaCl test solution mixture was discarded and the dishes were filled with 100 ml of fresh test solution. Large clumps of eggs and excess eggs were also removed at this time.

The number of eggs in each dish was obtained using a hand counter and a grid placed under the dishes to prevent counting eggs more than once. Test solutions were changed daily and dead eggs, often called "ringers," were also removed on a daily basis. This procedure prevented the buildup of bacteria or fungus.

When toxicity tests were performed inside the glove box, eggs were spawned on the outside in 250 ml Nalgene bottles, the top was placed on the bottles, and they were put into the glove box through the sphincter port. This procedure required approximately one minute from the time eggs were stripped from the female until they were placed into the test solution.

In natural waters, eggs are first deposited by the female, and subsequently fertilized by the male. The ideal situation for these toxicity experiments would have been to strip eggs from the female into the test solution then add the sperm. However, the glove box imposed physical restraints and required a delay period between when the eggs were stripped and when the sperm was added.

In the process of impregnation, an egg is penetrated by a spermatozoon through an opening known as the micropyle. Almost immediately,

the opening is blocked by further entry of spermatozoa by a cortical reaction inside the egg.⁹⁷ An experiment was performed to determine the effectiveness of fertilization as a function of the time between stripping the eggs and adding the milt. These data are illustrated in Figure 17. In the test group the addition of sperm was delayed for various intervals of time while in the control group sperm was added immediately after stripping the eggs. The results of this experiment indicate that the most effective fertilization occurs when milt is added within a few seconds after the eggs are stripped from the female. Based on these data, it was decided that eggs should be fertilized before they were put into the glove box.

Attempt to Delay Spawning

The spawning season for *C. carpio* may extend over a three month period during years when water temperature and other environmental conditions are favorable. However, it was desirable to have ripe male and female carp available for toxicity tests for longer than three months if possible. Therefore 20 carp were collected during early April and placed in 1 m deep x 3 m diameter pools in which water temperature was maintained at approximately 13°C. On June 10, the water temperature was slowly raised over a period of 14 days to induce spawning activity.

This attempt to delay spawning was effective in producing ripe males through July; however, no ripe females resulted from the experiment. Although there are reports in the literature that carp spawning activity has been delayed successfully, there are several reasons

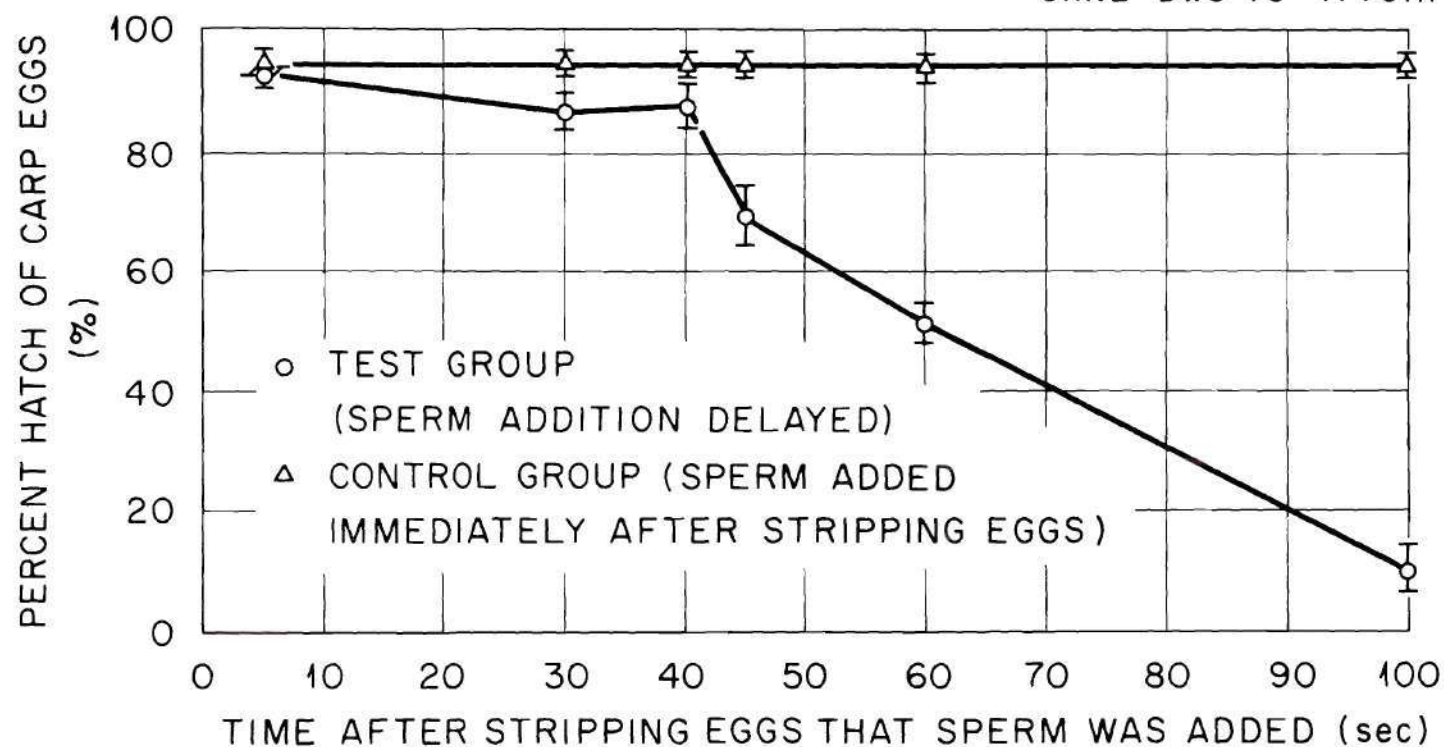


Figure 17. Percent Hatch of Carp Eggs as a Function of the Time Between Stripping the Eggs and Adding the Sperm

attributed to the failure to produce ripe males and females in this experiment. The most likely reasons were that wild carp did not adjust well to the limited space available in the pools and a suitable spawning habitat was not present. Approximately one-half of the specimens jumped out of the pool and died before July 1. In addition, the change from a natural diet to a laboratory diet may have interrupted normal sexual maturity.

Laboratory Spawning of *Pimephales promelas*

Technique for Obtaining *P. promelas* Eggs

It was necessary to design a system which would provide a plentiful supply of freshly fertilized *P. promelas* eggs for use in toxicity experiments with plutonium and uranium. Niazi¹⁰¹ reported that he was not successful in fertilizing fathead minnow eggs by stripping eggs and milt from ripe females and males. Therefore a natural system which allowed the eggs to be laid and fertilized by the fish was desirable. Eggs could then be checked to determine the stage of development. With the aid of the biological clock for *P. promelas* outlined in Chapter II (p. 47), those eggs which were not past the blastula stage were used for toxicity tests and older eggs were discarded.

Several techniques as well as two strains of fish were evaluated to provide a reliable source of *P. promelas* eggs. One strain was obtained from a local fish bait dealer. The other strain was obtained from the Environmental Protection Agency, National Water Quality Board Stock at Duluth, Minnesota.

The fathead minnow lays eggs in clusters, usually one egg deep, on the underside of objects. Eggs are attached by an adhesive coat and may be removed from the surface with a spatula. This procedure, however, is not recommended due to potential physical injury to the eggs.

Nesting sites were constructed by cutting 7 cm (ID) plastic pipe in half and in lengths of 8 cm. The inside of the pipe was covered with a piece of 1 mm thick translucent polyethylene. The polyethylene was attached to the bottom edges of the pipe with plastic tape. Several other materials to cover the inside of the pipe were tested. Among these were teflon, acetate, and aluminum foil. Each of these had disadvantages of either being expensive, toxic to the fish, or not acceptable by the fish as a satisfactory surface on which to build a nest and polyethylene was used in subsequent work.

The use of polyethylene to cover the underside of shelters has several distinct advantages. This material is flexible and assumes the shape of the pipe. The polyethylene can be easily cut with a pair of scissors to provide a test group of eggs and a control group of eggs. Because this material is translucent, fresh eggs may be observed under a microscope without removing them from the polyethylene.

The procedure followed to obtain eggs is illustrated in Figures 18 and 19. The polyethylene was attached to the shelter, Figure 18(a), and placed in a living stream or aquarium, (b). The male fathead prepared the nest for the female (c) and guarded the eggs attached to the top of the shelter (d). The polyethylene was removed from the shelter after the eggs were laid and excess polyethylene was discarded, Figure

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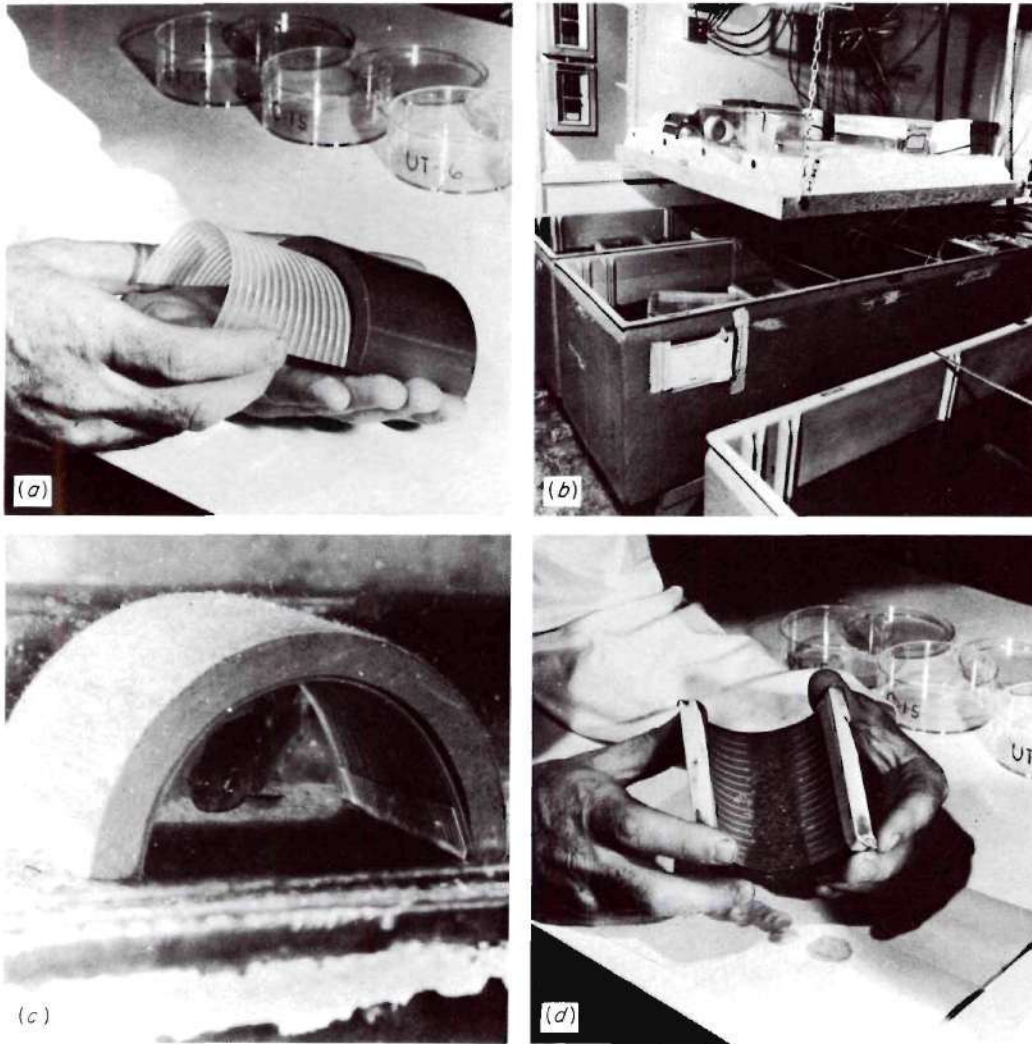


Figure 18. Technique Followed to Obtain Fathead Minnow Eggs

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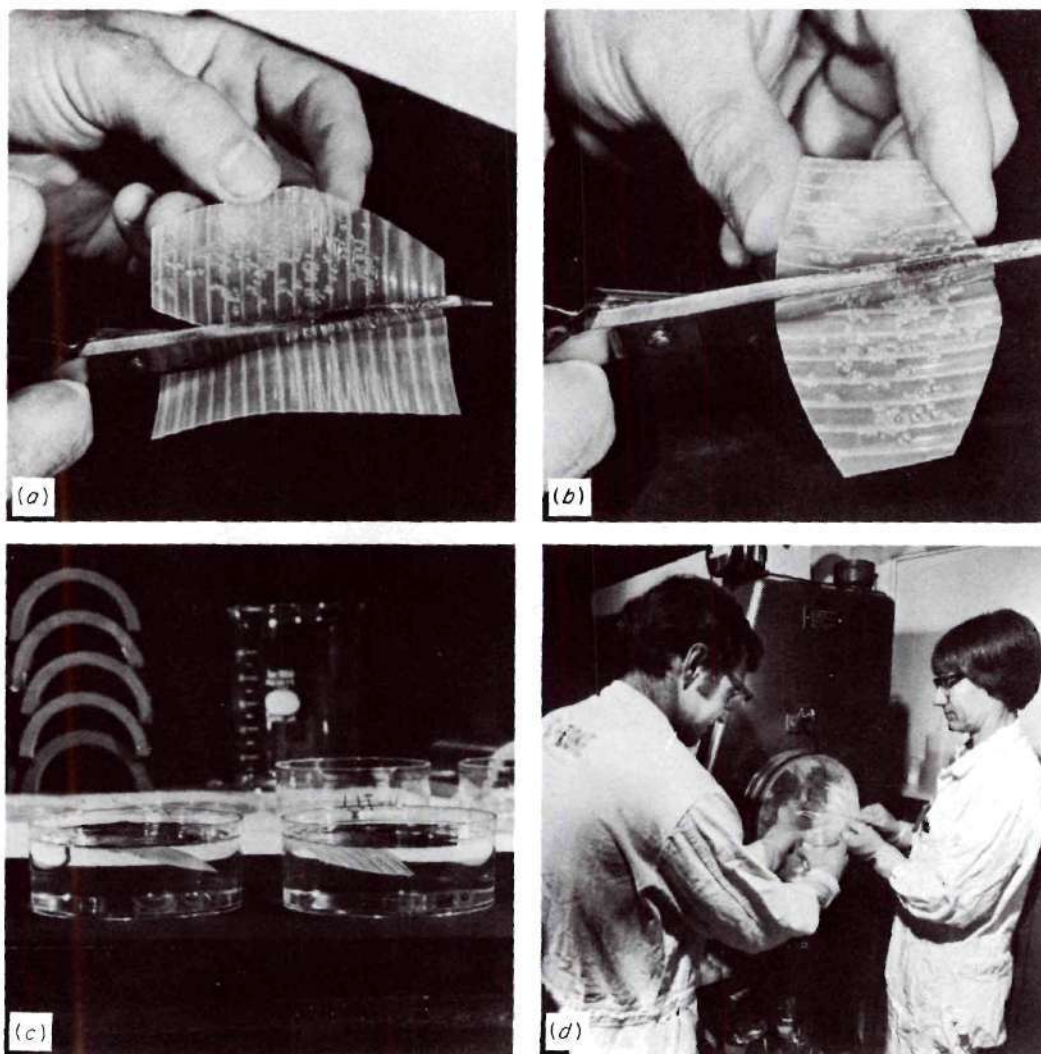


Figure 19. Technique Followed to Obtain Fathead Minnow Eggs

19(a). The group of eggs was cut into two pieces, each piece containing approximately the same number of eggs (b). The two pieces of polyethylene were floated in spring water in plastic dishes with the eggs facing downward (c) and, if necessary, transferred to the glove box (d).

Three different sized containers were used for holding the parent fathead minnows. These were 210 x 54 x 54 cm living streams containing 470 liters, 90 x 47 x 47 cm aquaria containing 170 liters, and 60 x 30 x 30 cm aquaria containing 32 liters. The small aquaria ultimately yielded the most eggs. In addition, the 60 x 30 x 30 cm aquaria were the easiest to maintain. A total of eight aquaria, each containing approximately four males and six females, provided most of the eggs for this study.

Lighting, Temperature, and Feeding Regime

It was determined that the type of food, the quantity of food administered at each feeding, and the regularity of feeding were significant factors influencing egg production. Breeders were given trout starter mash, frozen adult *artemia*, and live newly hatched *artemia* daily. The fish were fed all they would eat in the morning and in the afternoon on weekdays and once each weekend day. Excess food seemed to reduce egg production.

Standard 40-watt fluorescent lamps at normal intensity were operated by a timer to provide 16 hours of light and 8 hours of darkness. The water temperature was maintained at 25°C. Although most aquaria were equipped with aerators, egg production in those aquaria

with no aeration was approximately the same as in aquaria with aeration. Each aquarium had a continuous spring water flow.

No significant differences were observed between the number of eggs produced, the percent of eggs hatching, or the survival of larvae from the EPA strain and those obtained locally. Data showing egg production and hatchability for 2446 eggs are listed in Table 9. These data were recorded over a period of 35 days. Only eggs which were taken from the nest at the blastula stage or earlier are included. Groups of eggs in which fungus developed and affected the percent of

Table 9. Fecundity and Percent Hatch for *P. promelas* under Controlled Conditions

Number of Eggs Observed	Mean Number of Eggs per Spawning	Development Temperature (°C)	Number of Abnormal Larvae	Percent Hatch (%)
2446	98	23.5	2	89.8

eggs hatching are not listed. These same criteria were also followed during toxicity tests, i.e., eggs which had passed the blastula stage of development were not used for toxicity tests. Eggs which had significant mortalities among both the control group and the test group were discarded and the test was repeated.

Dead eggs were removed at least daily and the test solution was also changed daily. Because fathead minnow eggs require approximately six days to hatch, it was more difficult to prevent the spread of

fungus or damage by bacteria. Once fungus developed, it was often more effective to remove all of the eggs in the area of the fungus.

Plutonium and Uranium Chorion Penetration Experiments

Quantitative Analyses

One major objective of this study was to investigate the biochemical behavior of plutonium and uranium associated with the developing embryos of fish. This included determining the quantity of these elements passing through the chorion and depositing in the egg contents. The information permitted an estimate of the dose to the egg from alpha radioactivity emitted by these elements.

Carp eggs were placed in solutions containing approximately 10^{-2} $\mu\text{Ci/ml}$ of ^{238}Pu or ^{233}U . Eggs were initially placed in the radioactive solutions at four stages of maturity: (1) immediately after fertilization, (2) 2 hours after fertilization, (3) 24 hours after fertilization, and (4) 48 hours after fertilization. Eggs that were not immediately exposed to ^{238}Pu or ^{233}U were allowed to develop in filtered spring water until they were placed in the radioactive solution. In order to evaluate the accumulation of radioactivity by the egg, samples were taken and analyzed at five intervals for each of the four stages of maturity listed above: (1) 2 hours, (2) 8 hours, (3) 24 hours, (4) 48 hours, and (5) 72 hours. Quantitative measurements of chorion penetration were made by analyzing radioactivity associated with three types of samples — total egg, egg content, and egg membrane. The procedure outlined above is illustrated in Figure 20.

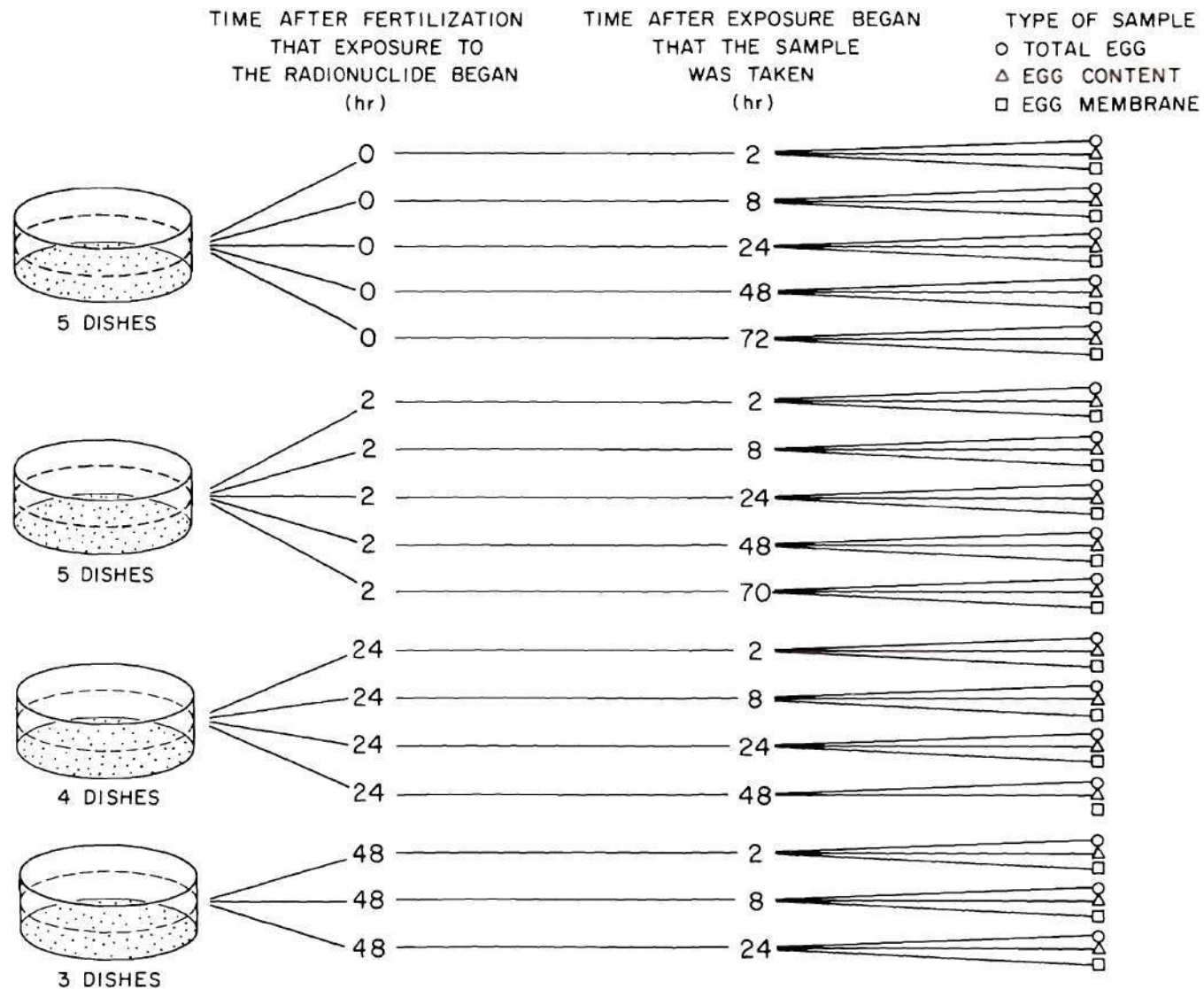


Figure 20. Samples Taken for Quantitative Penetration Experiments

This technique provided three specific types of information. First, it showed the accumulation of plutonium or uranium by the egg as a function of the time that the egg had been exposed to radioactivity. Second, this method demonstrated how chorion penetration was affected by the stage of development of the egg. Finally, the three types of samples, total egg, egg content, and egg membrane, helped to quantify radioactivity inside the egg and radioactivity adsorbed to the egg membrane.

Prior to taking samples, eggs were transferred to a clean plastic dish and rinsed with distilled water. This procedure was followed to remove debris attached to the chorion and also to remove radioactivity not permanently fixed to the outside of the egg. The egg content was separated from the membrane by placing the egg on Whatman No. 42 filter paper (product of W&R Balston Limited), gently holding the egg with fine forceps, and bursting the egg with a dissecting needle. The egg content was absorbed by the paper and the membrane was retained by the tweezers for separate analysis. After the contents of approximately 50 eggs had been absorbed onto a small section of the paper usually about 4 cm^2 in area, excess paper was removed and the small piece containing the absorbed egg contents was put into a 15 mm x 175 mm test tube for future analysis. Similarly, the membranes were put into another test tube. In addition, a sample consisting of 50-100 total eggs was placed into a third tube.

Prior to analysis by gross alpha counting, samples were transferred to 30 ml beakers. Test tubes were rinsed with 1 M nitric acid

to remove any plutonium or uranium that remained in the bottom of the tube. Total eggs, egg membranes, and egg contents absorbed by the filter paper were digested in a solution of 10 ml of concentrated nitric acid and 5 ml of distilled water maintained at low heat. Several drops of 30% hydrogen peroxide were also added at one hour intervals. The pyrex beakers were covered with watch glasses to reduce evaporation, and distilled water or concentrated nitric acid were added periodically to keep the total volume at 15 ml. Digestion of samples containing only total eggs or membranes could be completed in 2-4 hours; however, digestion of the egg contents that had been absorbed onto filter paper required 6-8 hours. When the solution in a beaker became clear, the watch glass was removed and the volume was evaporated to approximately 2.0 ml. The solution was then transferred to a 5.0 cm (ID) stainless-steel planchet. Beakers were rinsed with 2-3 ml of 1.0 M nitric acid. The liquid in the planchet was evaporated to dryness before the sample was heated by flame to fix the radionuclide to the planchet. Samples were analyzed for gross alpha activity using a gas-flow proportional counter.

Autoradiograph Materials and Procedures

Slide Preparation. Autoradiographs were made of egg sections to determine the distribution of plutonium and uranium inside the egg. Two or three eggs were taken from each group exposed to ^{238}Pu or ^{233}U in the quantitative penetration experiments and placed into a 10 x 72 mm test tube. Subsequently eggs were embedded in a polyacrylamide gel following the procedure reported in the literature by Orlowski et al.¹³³

Eggs were preserved in the gel under refrigeration until they were prepared for sectioning.

The gel was removed from the tube by pressure applied with a syringe filled with distilled water. Eggs and gel were frozen in liquid nitrogen and 16 μ thick sections cut with a microtome. Approximately eight sections were placed onto each slide that had been coated with Haupt's Gelatin Affixative. Slides were dried for 30 minutes at 35°C.

Autoradiograph Procedure. The liquid emulsion dipping method discussed by Gude¹³⁴ was followed to prepare autoradiographs of slides. Kodak Nuclear Track Emulsion, type NTB, was melted in a water bath at 42°C in a dark room. Slides were dipped into the emulsion three times to assure uniform coating and dried in a vertical position to allow excess emulsion to drain away. Following this, slides were placed in boxes, sealed to prevent exposure from light, and kept under refrigeration to prevent deterioration.

Optimum exposure period was determined by developing two or three slides periodically and observing the number of alpha particle tracks that had formed. Following exposure, autoradiographs were developed in Kodak D-19 developer and Kodak fixer. Slides were stained by dipping for three minutes into a solution containing 1.0 g of hematoxylin, 0.6 g of aluminum ammonium sulfate, and 1.2 g of mercuric oxide in 400 ml of water followed by one minute in an aqueous solution of 0.5% eosin. Slides were then dried at 35°C. Photographs were prepared of selected slides using Kodak Panatomic X film and a photomicroscope.

Toxicity Experiments

The second objective of this study was to determine the radiological and chemical toxicity of plutonium and uranium to developing fish embryos. There have been no previous conclusive experimental investigations to establish an upper limit of toxicity to fish eggs from exposure to radiation effects or chemical effects of plutonium or uranium.

Procedure for Toxicity Experiments

Toxicity tests were performed employing the eggs of carp and fathead minnows that were obtained following the procedure outlined in the preceding sections. Eggs were placed into the test solution immediately after fertilization for carp and between the one cell stage and early blastula for fathead minnows. Developing embryos were not disturbed during exposure except to change the test solution daily and to remove embryos that had died. A record was maintained of the number of eggs removed prior to hatching and the number hatching. Abnormal larvae were also observed.

Usually three groups of eggs, each group in a separate dish, were tested for each concentration of solution. However, in some cases where the quantity of isotope available was restricted, for example, ^{244}Pu , only one or two groups per treatment concentration of eggs were examined at the higher concentrations.

Procedure for Survival Experiments

Larvae were removed from the test solution with an eye dropper after they hatched. Approximately 100 larvae were retained to observe

survival following hatching for most concentrations. These fish were transferred to 13 x 20 x 33 cm plastic pans and maintained at $24 \pm 1^\circ\text{C}$ in an environmental chamber. Lighting was regulated in the chamber to provide 16 hours of light and 8 hours of darkness. Each pan was equipped with a forced air supply through an airstone. Each pan contained five liters of spring water and additional water was added as necessary to make up for losses by evaporation. Dead larvae were removed from the pans at approximately three day intervals and the number removed was recorded. Live larvae were counted at three week intervals and the water in the pans replaced with a fresh supply.

Carp and fathead minnow prolarvae were not fed until 7-10 days after hatching. During this period the yolk sac is being absorbed. Postlarvae were fed a diet of powdered freeze-dried *artemia* sprinkled on the surface of the water. During the first 30 days after hatching larvae were fed at 3 or 4 day intervals. As growth progressed the intervals were decreased to daily feedings.

Toxic Substances Evaluated

In addition to evaluating the toxicity of ^{238}Pu , ^{244}Pu , ^{232}U , ^{235}U , and ^{238}U , toxicity tests were also performed with trisodium citrate, $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$, and potassium fluoride, KF. Upper limits for citrate toxicity were needed because the citrate complexes of plutonium and uranium were used. Potassium fluoride toxicity to developing eggs was also investigated because of the potential significant importance fluorides will have as a common chemical form of plutonium and uranium in the nuclear fuel cycle.

CHAPTER IV

RESULTS

Egg Chorion Penetration by Plutonium and UraniumQuantitative Experiments

Plutonium. Appendix B lists the results of plutonium penetration experiments in which the egg content, the egg membrane, and the total egg were analyzed for ^{238}Pu uptake. The concentration of the ^{238}Pu test solution was $2 \times 10^{-2} \mu\text{Ci/ml}$. Three samples were prepared from eggs taken from each dish—egg contents, egg membranes, and total eggs. The concentration of activity in the carp egg was calculated by dividing the activity per egg by the egg volume, $4.2 \times 10^{-3} \text{ ml}$ (assuming a mean egg diameter of 2.0 mm). The concentration factor is a unitless value calculated by dividing the activity concentration in the egg volume by the activity concentration in the test solution. A concentration factor of one implies that the concentration in the egg contents is the same as the concentration in the test solution.

Figure 21 graphically illustrates the data presented in Appendix B for ^{238}Pu uptake by the total egg. It was concluded from Figure 21 that plutonium uptake by the total egg occurred continuously throughout development for eggs placed in solution immediately after fertilization and two hours after fertilization. Although not included in Appendix B, a similar set of data for total egg uptake of ^{238}Pu were obtained by

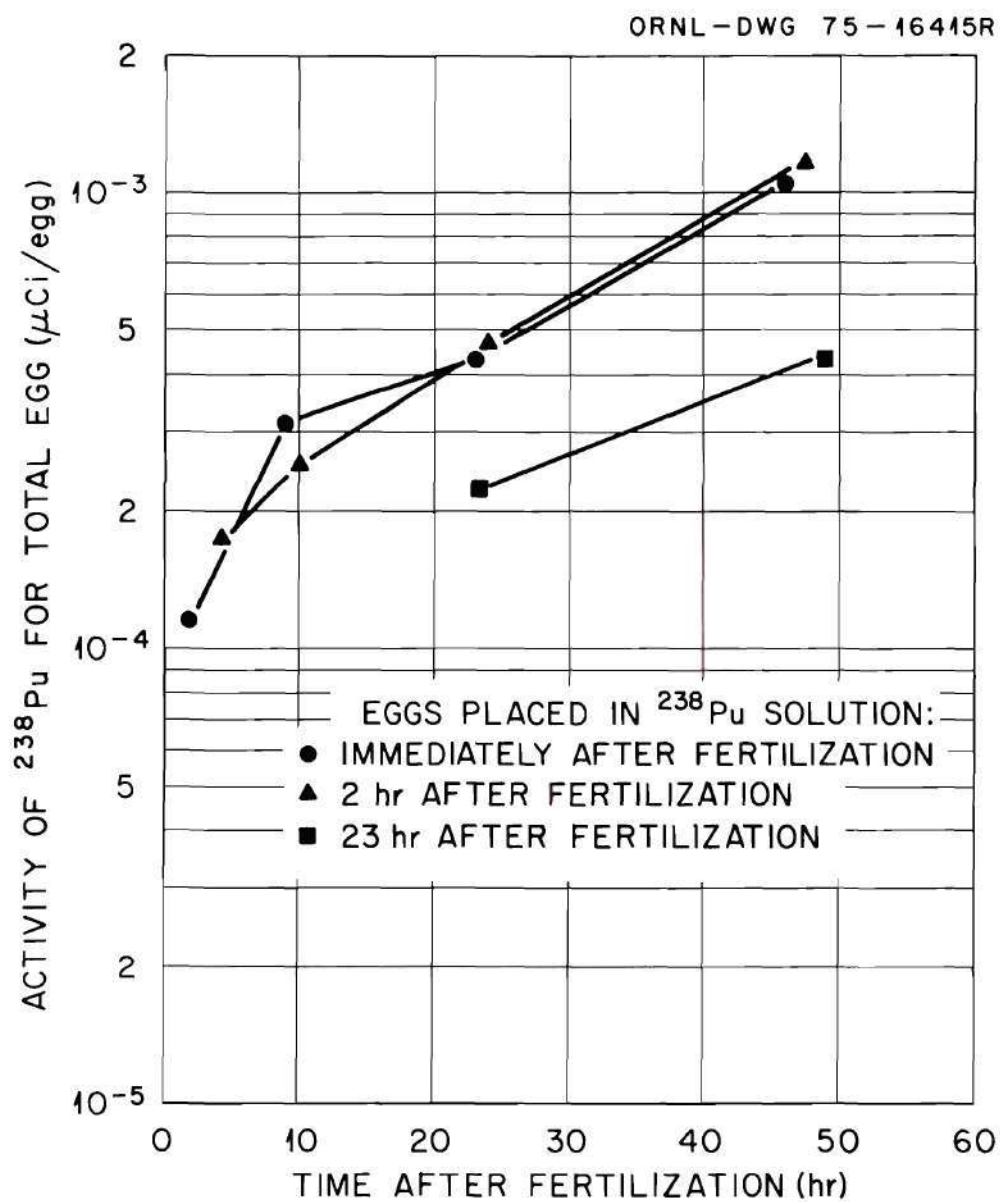


Figure 21. Accumulation of ^{238}Pu by Carp Eggs

rinsing the eggs in EDTA chelating agent prior to placing them in the test tube. This procedure was followed to remove plutonium loosely adsorbed to the egg membrane. These data are depicted in Figure 22. Although the EDTA was effective in slightly reducing the total activity associated with each egg, the same pattern for uptake was observed, namely, a constant accumulation of plutonium.

Figure 23(a) shows the activity of ^{238}Pu in the contents of the egg. Uptake by the egg content continued throughout development at approximately the same rate regardless of the time at which the egg was first exposed to the plutonium. Figure 23(b) illustrates the percent of the total egg activity that is associated with the egg contents. Although the egg continuously accumulated plutonium throughout development, the fraction of activity on the membrane and in the contents remained at a relatively constant value. A mean value of 33% of the total egg activity was inside of the egg and 67% remained with the chorion.

The concentration factor for ^{238}Pu in carp eggs exposed to $2.0 \times 10^{-2} \mu\text{Ci/ml}$ is illustrated in Figure 24. Eggs placed into the test solution 0, 2, and 23 hours after fertilization exhibited similar uptake characteristics. Within 10 hours after the eggs had been exposed to plutonium, the concentration in the egg contents was greater than the concentration of the test solution. At 50 hours after fertilization, the concentration factor for eggs exposed immediately had increased to a value of approximately three. Several conclusions were drawn from these data. First, plutonium uptake by fish eggs occurs continuously

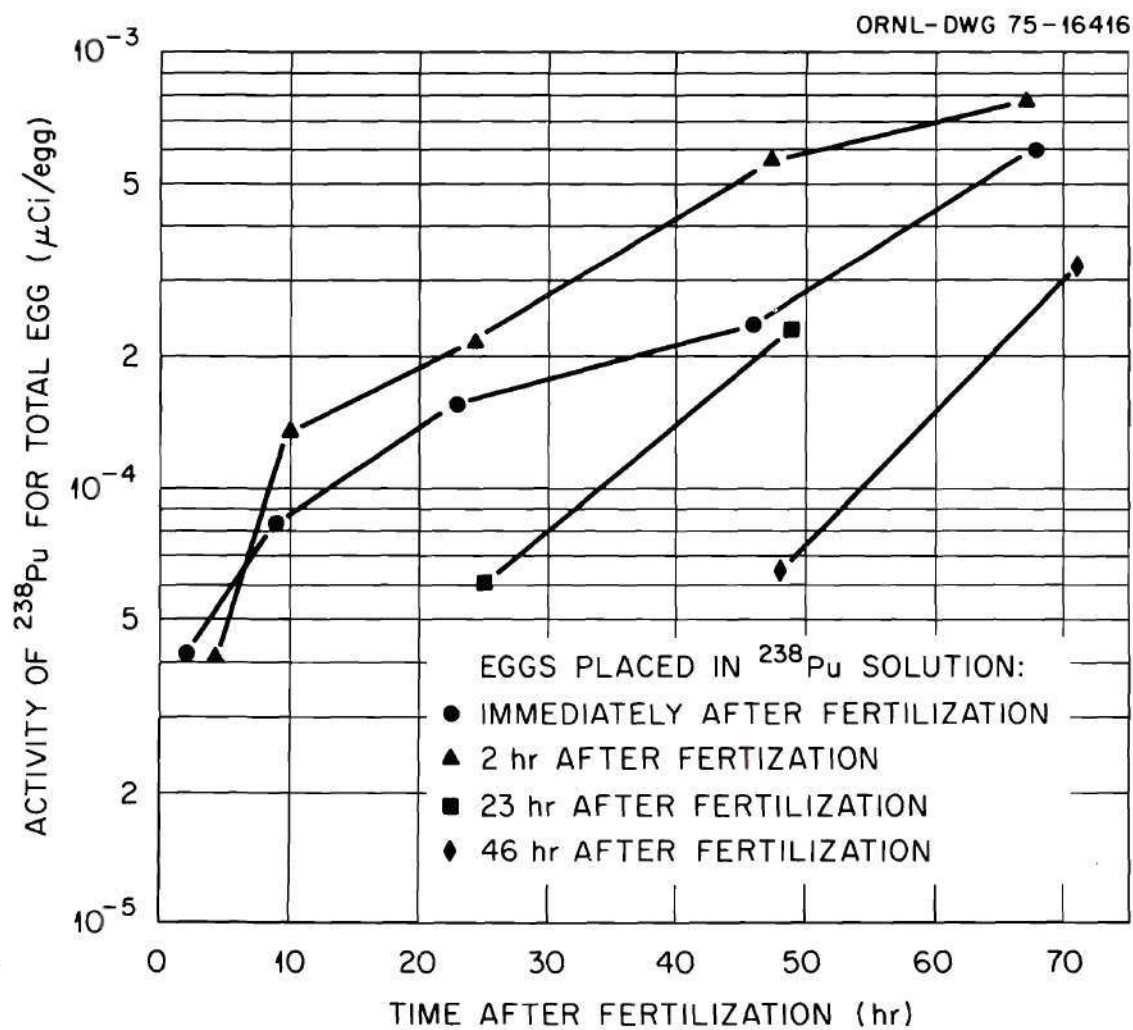


Figure 22. Accumulation of ^{238}Pu by Carp Eggs
(with EDTA Rinse)

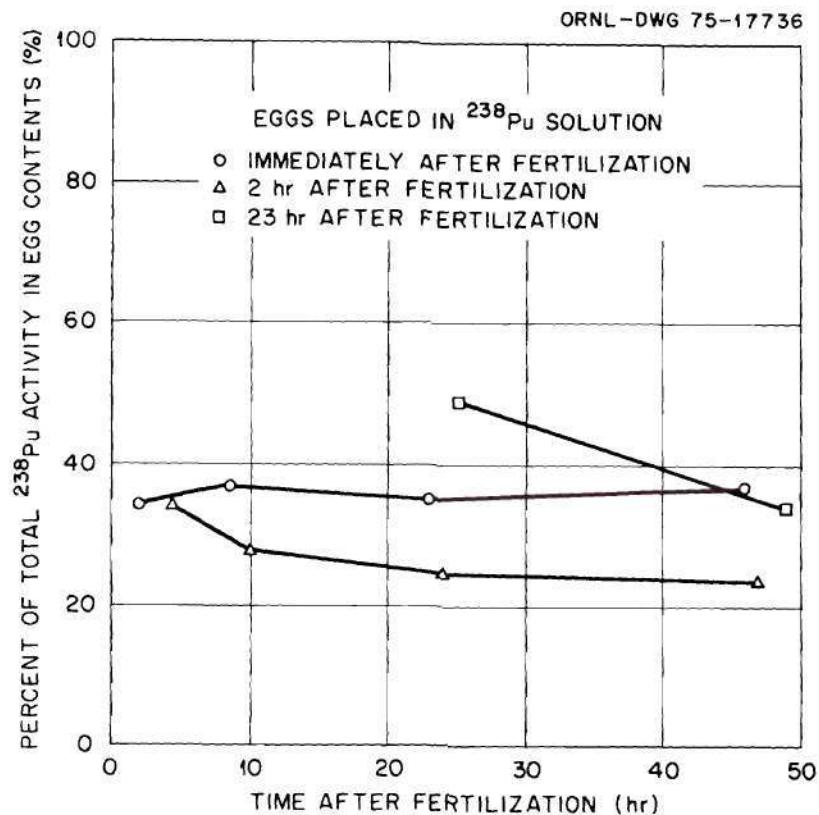
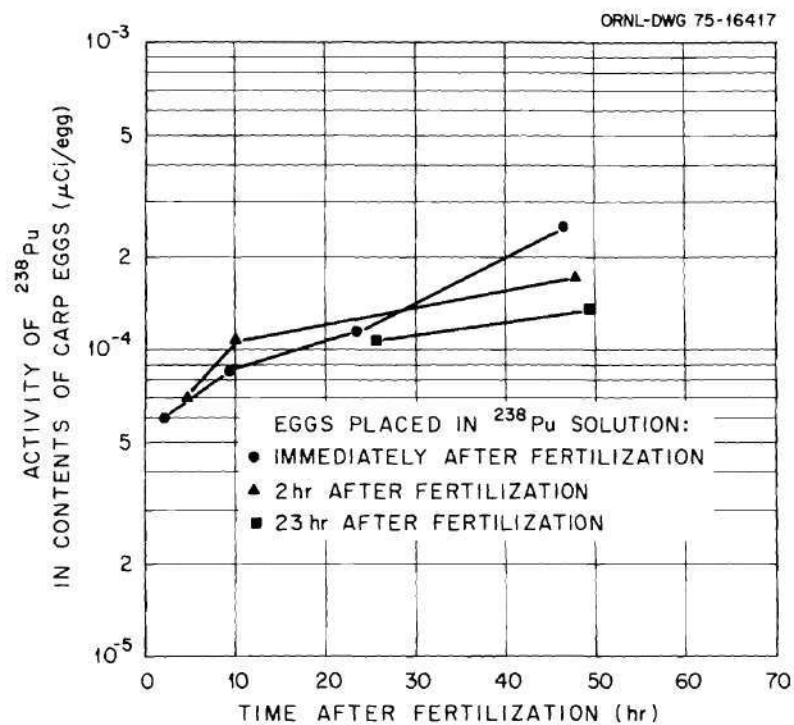


Figure 23. ^{238}Pu in the Contents of Carp Eggs:
 (a) Accumulation During Development,
 (b) Percent of Total Egg
 Activity in the Contents

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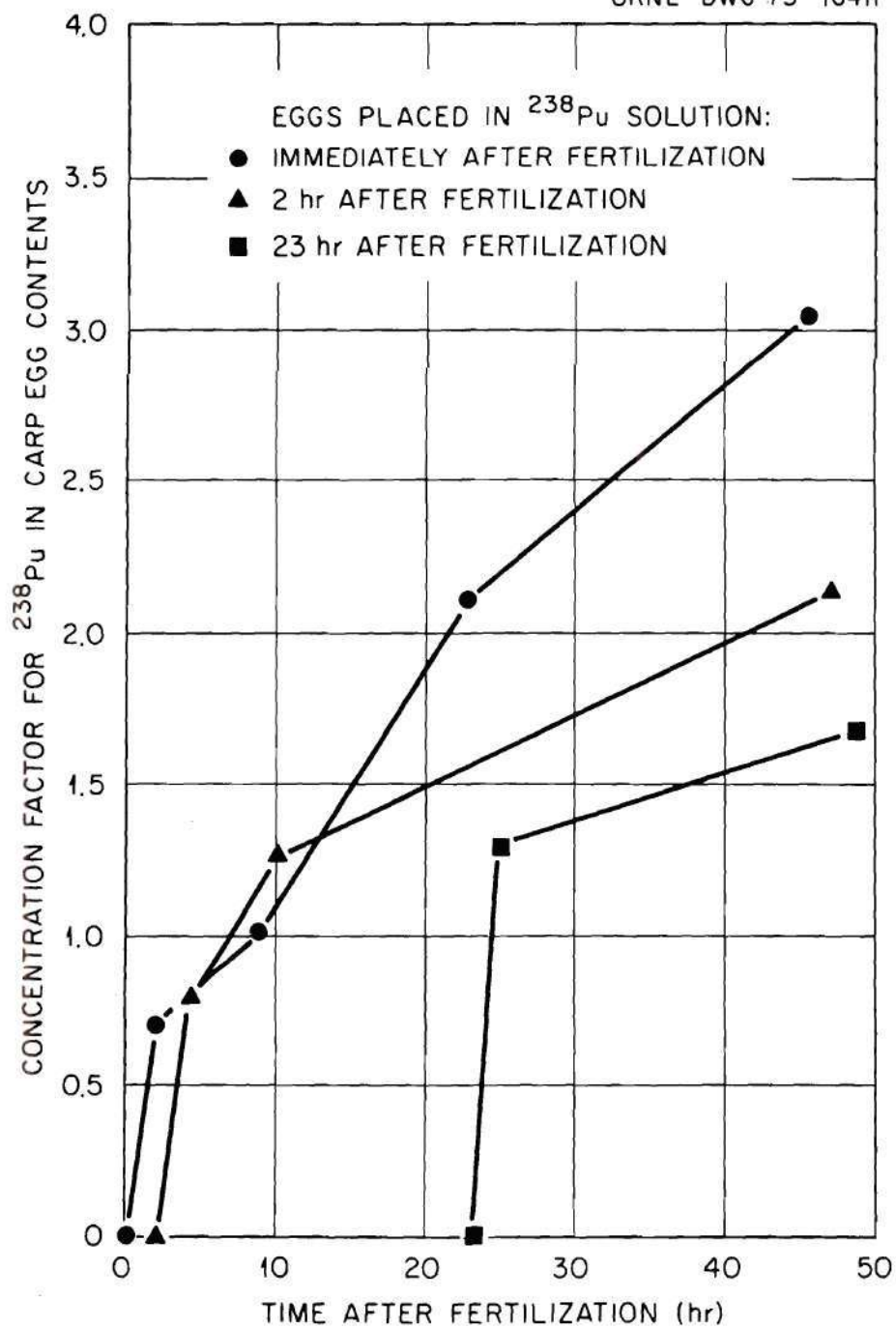


Figure 24. Concentration Factor for ^{238}Pu in the Contents of Carp Eggs

throughout development rather than before water hardening of the chorion. Second, the rate of plutonium uptake by the egg contents is similar for eggs placed into the test solution at 0, 2, and 23 hours after fertilization. Third, the rate of plutonium uptake decreases as egg development progressed indicating that a saturation level would be reached for eggs which had an embryological development period longer than several days.

The concentration of plutonium in the egg contents was a function of the length of time that the eggs had been exposed to plutonium. Likewise, the absorbed dose rate from alpha radiations emitted in the egg was a function of the exposure period. In order to calculate the dose rate at any given time during development, a function representing the uptake of plutonium by the contents of the egg had to be determined. Figure 25 shows a least squares fit of the function $y = c\sqrt{x}$ where c is a constant. The function was obtained empirically and, with $c = 0.44$, fits the data well for eggs exposed immediately after fertilization.

Uranium. The results of quantitative experiments to determine the penetration of uranium through the chorion of carp eggs are included in Appendix B. Concentration of ^{233}U in the test solution was $1.2 \times 10^{-2} \mu\text{Ci/ml}$. Three samples were again prepared — egg contents, egg membranes, and total eggs. The technique used to obtain samples was identical to that for plutonium.

Figure 26 depicts the uptake of ^{233}U by the total egg as a function of the time after fertilization. Unlike the graphical plot of similar data for plutonium in Figure 21, it is difficult to discern any regular pattern of uptake for uranium. In general, however, it is

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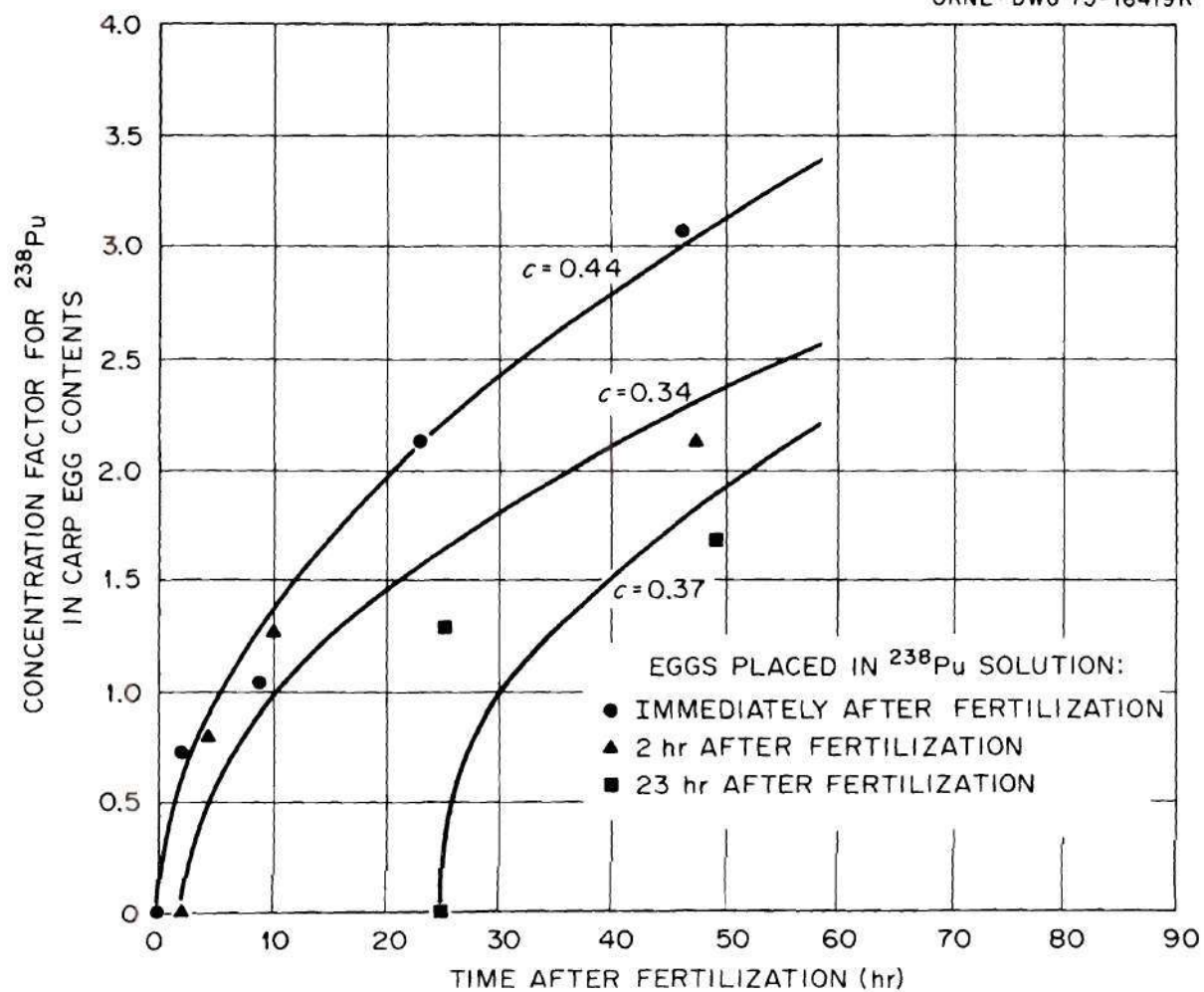
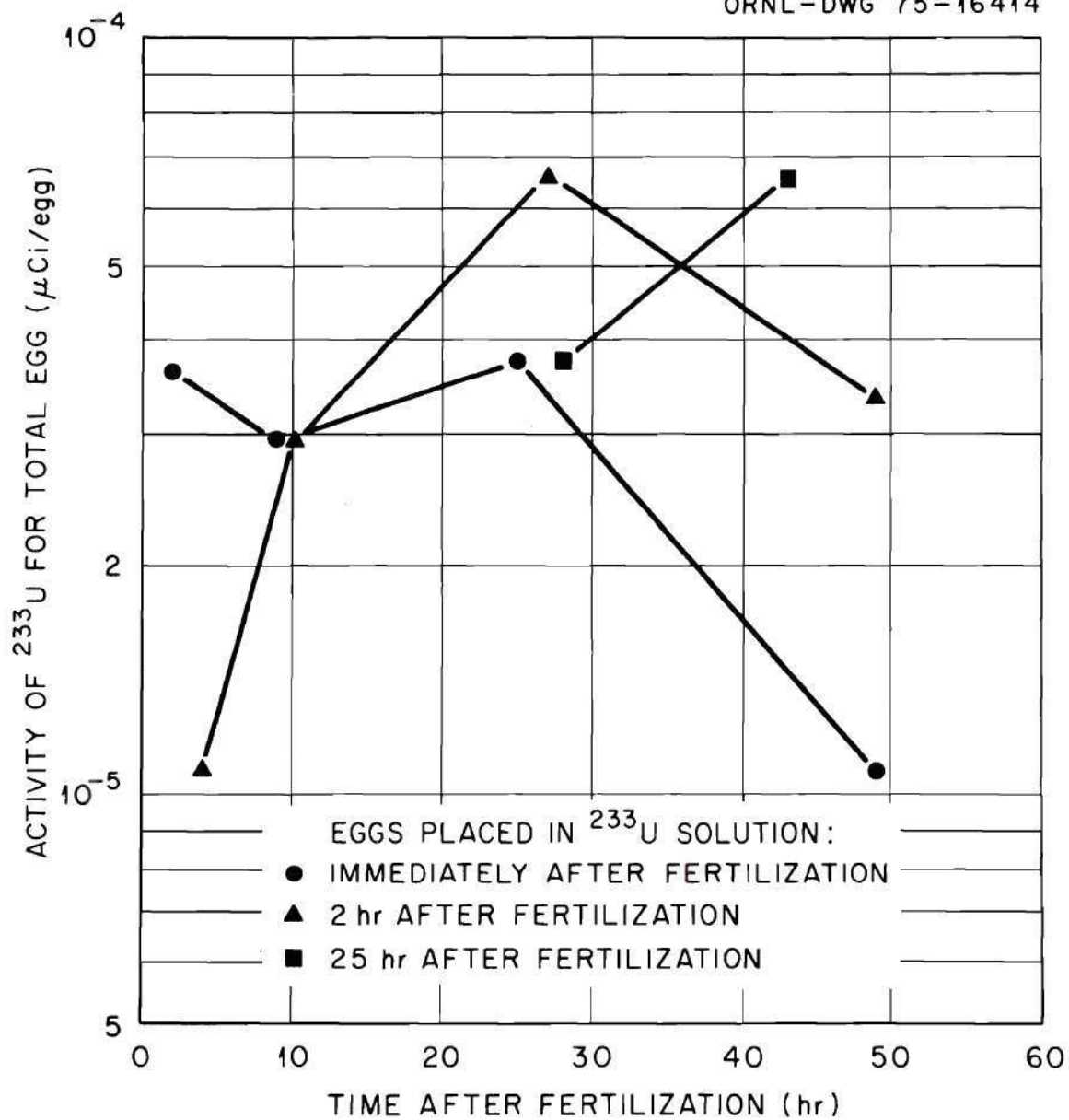


Figure 25. Least Squares Fit of the Concentration Factor
for ^{238}Pu in Carp Egg Contents

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Figure 26. Accumulation of ^{233}U by Carp Eggs

noted that the total activity per egg was less for uranium than for plutonium. The activity of ^{233}U in the contents of carp eggs and the percent of the total activity in the egg contents as a function of time after fertilization are well illustrated in Figures 27(a) and 27(b). In contrast to plutonium, a higher percent of the total egg activity was associated with the egg content rather than the membrane. Assuming that the percent of total activity in the egg contents remained approximately constant, a mean value of 60% was calculated.

The concentration factor for uranium in the egg contents as a function of the time after fertilization is displayed in Figure 28. For eggs exposed immediately, the concentration factor increased to a maximum of 0.8 then decreased to a value of 0.1 at 50 hours. Similarly, with eggs exposed later during embryological development, the concentration factor increased to a maximum then decreased again. The distribution of these data suggested that uranium was absorbed and later excreted by the embryo during development. When these data were analyzed the carp spawning season was over and it was not possible to repeat the experiments to verify the uptake pattern in Figure 28. Therefore, a series of experiments were performed to evaluate the uptake of ^{233}U using fathead minnow eggs as a test organism. The small size of the fathead egg made quantitative experiments in which the membrane and egg contents were separated difficult to perform and only uptake by the total egg was analyzed. Eggs were exposed to the ^{233}U test solution for varying periods of time before the sample was taken although exposure began before the blastula stage in each case. The

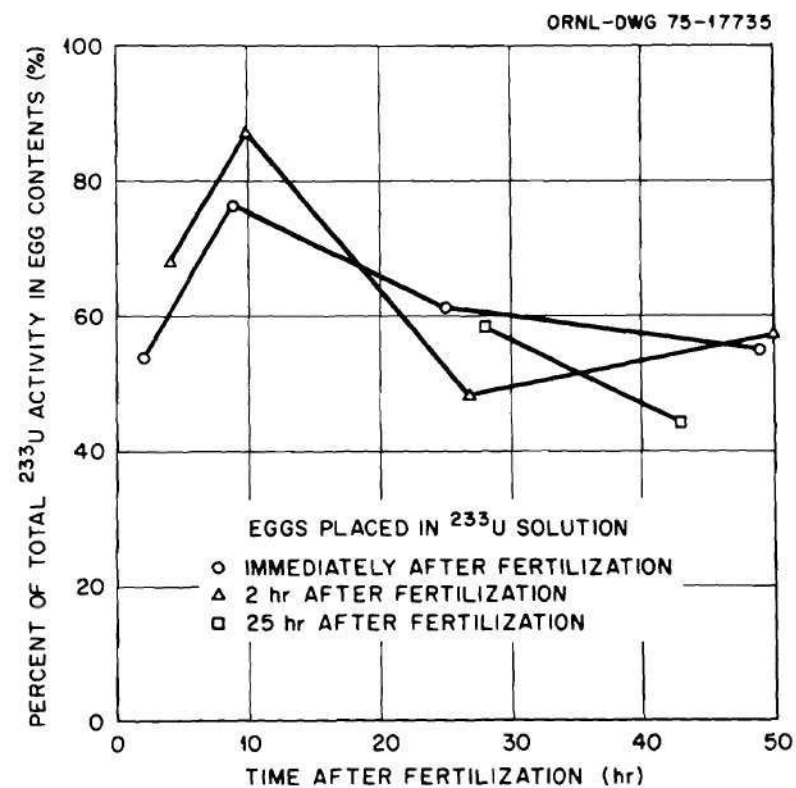
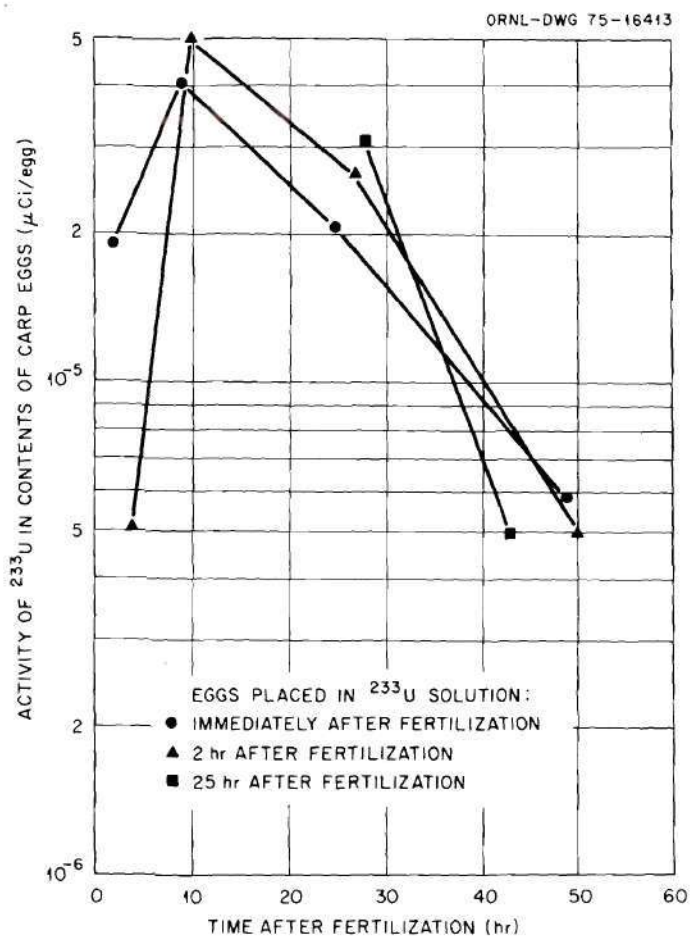


Figure 27. ^{233}U in the Contents of Carp Eggs:
 (a) Accumulation During Development,
 (b) Percent of Total Egg Activity in the Contents

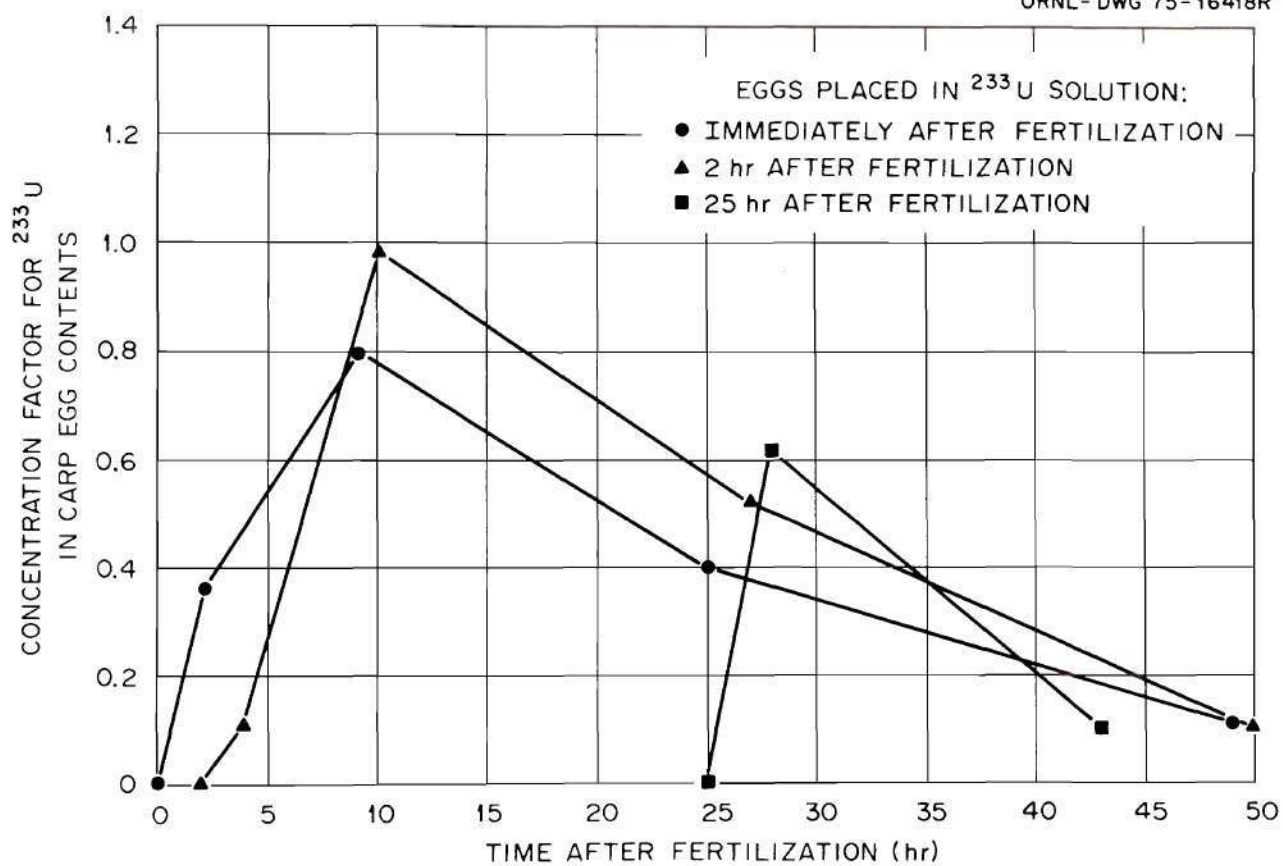


Figure 28. Concentration Factor for ^{233}U in the Contents of Carp Eggs

concentration factor was calculated by assuming 60% of the total egg activity was in the egg contents.

The results of this experiment are shown in Figure 29. These data suggest that the concentration factor did not vary considerably over the incubation period. A mean concentration factor of 0.38 is indicated in the figure.

Several conclusions were drawn from these data regarding the penetration of the chorion by ^{233}U . The concentration of uranium in the egg contents varied widely during embryological development as a result of physiological changes in the egg. These data indicate that uranium was not greatly accumulated by the developing fish egg. A mean concentration factor of 0.42 was calculated from the data in Figure 28 and a factor of 0.38 in Figure 29. These values were in good agreement. For dose calculations used later in this study it was assumed that the concentration factor for uranium in the egg contents was a constant value of 0.4 throughout development even though it was recognized that there appears to be a wide variation in the data in Figures 28 and 29. Insufficient data are available at this time to incorporate the uptake of uranium as a function of time into dose estimates.

Summary of Quantitative Data. The preceding discussions on plutonium and uranium penetration of the egg chorion are summarized below.

1. Plutonium uptake by the egg contents assuming uniform distribution

$$\delta(t) = \kappa\sqrt{t} \quad (1)$$

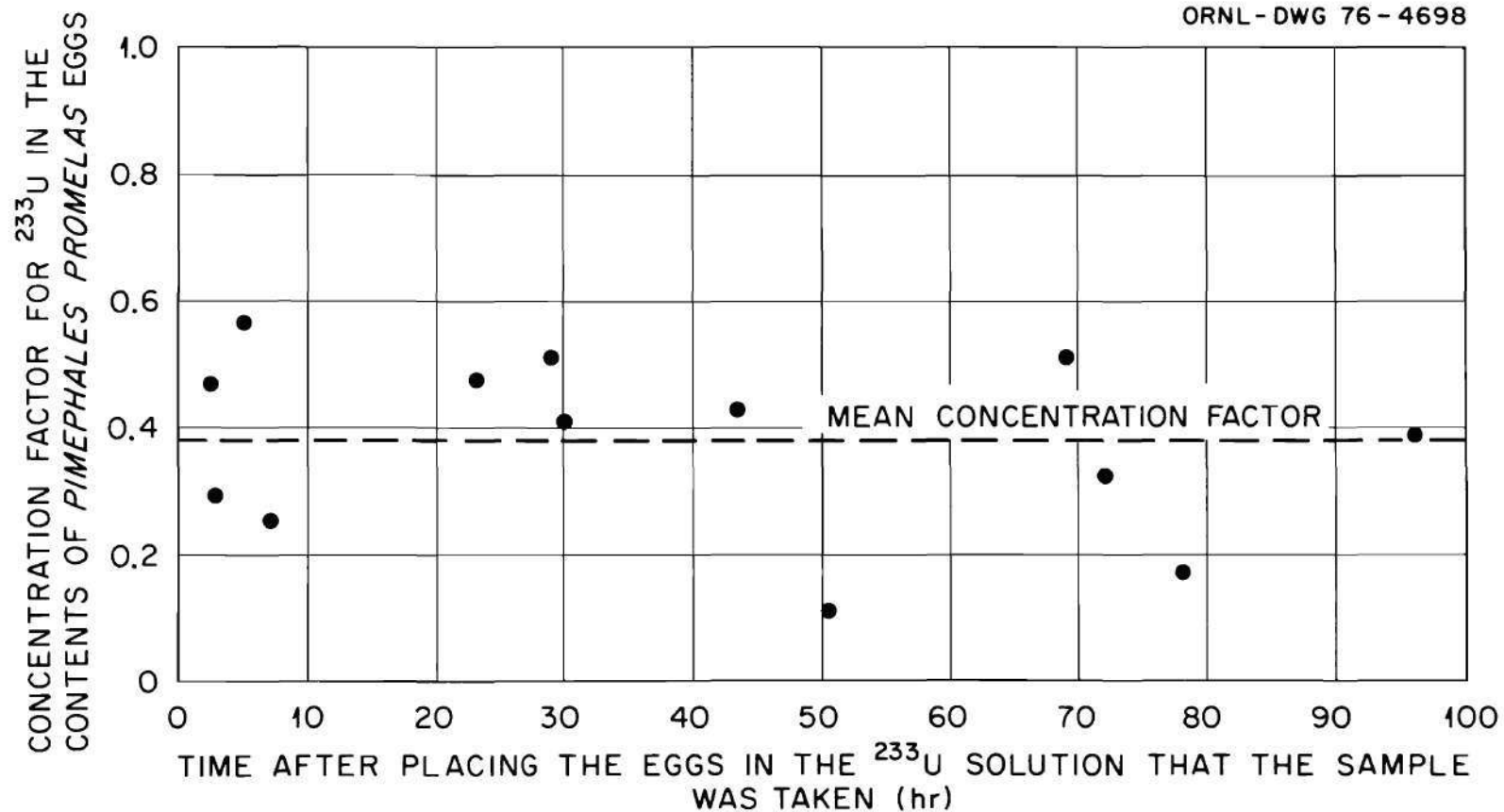


Figure 29. Concentration Factor for ^{233}U in the Contents of Fathead Minnow Eggs

where

$\delta(t)$ = concentration factor (dimensionless) for the
egg contents at time t ,

t = time after the eggs were placed into the test
solution, and

$\kappa = 0.44$.

2. Uranium uptake by egg contents assuming uniform distribution

$$\delta = 0.4 \quad (2)$$

where

δ = mean concentration factor for the egg contents.

Autoradiographs

Plutonium. Autoradiographs were prepared according to the procedures outlined in the preceding chapter. These autoradiographs confirmed that although a buildup of activity occurred on the chorion, plutonium also entered the contents of the egg and was uniformly distributed. The optimum exposure period was four days for plutonium autoradiographs.

Figures 30(a) and 30(b) depict autoradiographs made from carp eggs that remained in the test solution for two separate lengths of time. Both eggs were placed in the solution immediately after fertilization; in Figure 30(a) the egg was removed and embedded in gel after 23 hours and in Figure 30(b), after 68 hours. Each egg was rinsed with water prior to embedment. In Figure 30(a) the perivitelline space is

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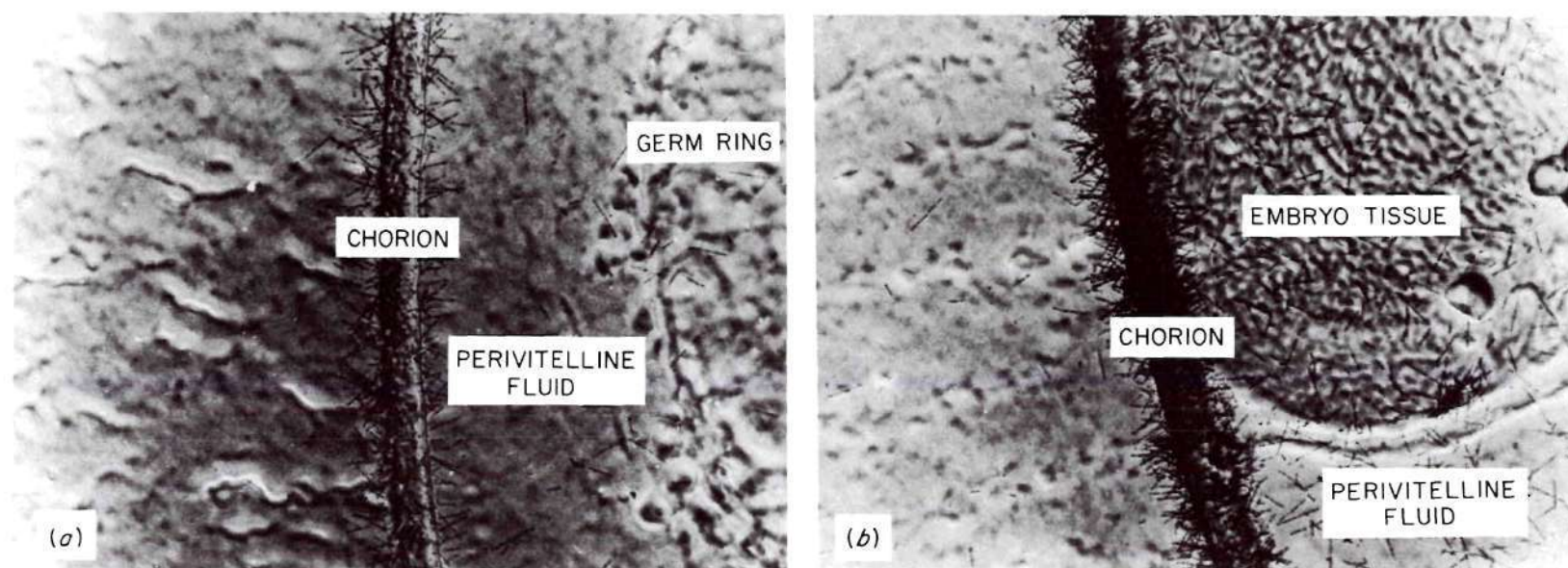


Figure 30. Autoradiographs Showing the Distribution of ^{238}Pu in Carp Eggs

visible as well as outlying layers of the germ ring. Plutonium activity is noted by the alpha particle tracks, most of which are located on the egg membrane. In Figure 30(b), the same distribution of activity is observed although more accumulation of plutonium has taken place both on the membrane and in the egg contents. Distribution inside the egg, however, is still relatively uniform.

Some alpha particle tracks were noted on the outside of the egg membrane in the embedding material. These tracks most likely resulted from plutonium which was mobilized before the acrylamide gel became firm. Autoradiographs were carefully scanned around the circumference to determine whether the microtome knife spread plutonium through the section during cutting. If the knife had redistributed the plutonium, it was predicted that one area of the embedding gel outside the membrane would have a high concentration of alpha particle tracks. This was not observed. Plutonium was found in the contents of eggs exposed to the test solution for all intervals and distribution was uniform throughout the perivitelline fluid, yolk, and embryo.

One characteristic of plutonium adsorption to the egg chorion was the formation of intense localized activity. These "hot spots" were observed on all of the plutonium autoradiographs. Figure 31 shows a typical hot spot on the outside surface of a carp egg. A study of the hot spots under high magnification clearly demonstrated that the points of intense localized activity were located on the outside of the chorion. Therefore the absorbed dose to the embryo from the alpha particle emissions at these points was insignificant compared to the

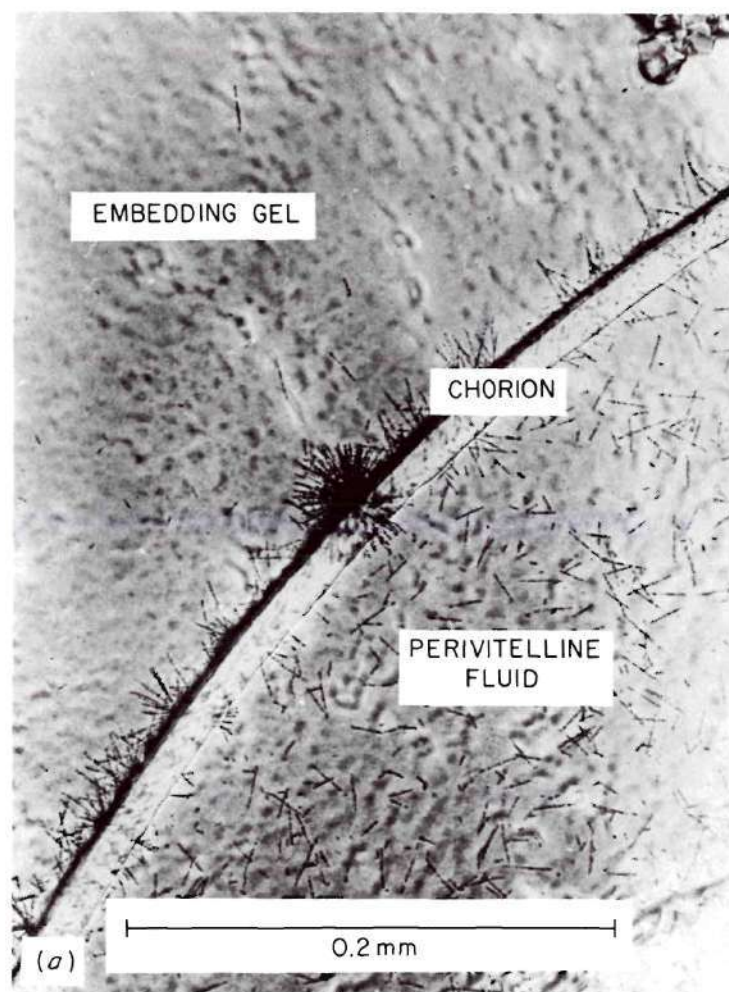


Figure 31. Plutonium Hot Spot on the Chorion

absorbed dose from alpha radioactivity originating inside the egg. Hot spots were not observed within the egg contents.

Several eggs that had been exposed to plutonium were rinsed with a 10% solution of EDTA chelating agent before they were embedded in gel. A count was made to determine the number of hot spots on eggs that had been rinsed with and without EDTA. A total of 31 hot spots were found on 25 egg sections made from eggs rinsed only in water while 14 hot spots remained on sections of eggs that had been rinsed in EDTA. These data confirmed that the points of localized activity were not permanently attached to the shell.

Uranium. Autoradiographs of egg sections that had been incubated in ^{233}U required a longer exposure period than egg sections incorporating ^{238}Pu . Most of the ^{233}U autoradiographs were developed between 50 and 60 days after the slides were dipped in the NTB emulsion. During this interval they were kept under refrigeration in light-tight boxes to minimize deterioration of the emulsion.

The distribution of uranium associated with the developing fish egg contrasted greatly with plutonium. Hot spots were not observed on egg sections containing ^{233}U . Uranium did not adsorb to the chorion as readily as plutonium. More important, uranium was not distributed equally throughout the egg contents but was localized primarily in the yolk.

Figures 32(a) and 32(b) are composite photographs showing 16 μ sections of carp eggs incubated in ^{233}U and ^{238}Pu . Each composite was prepared by joining seven individual photomicrographs originally taken

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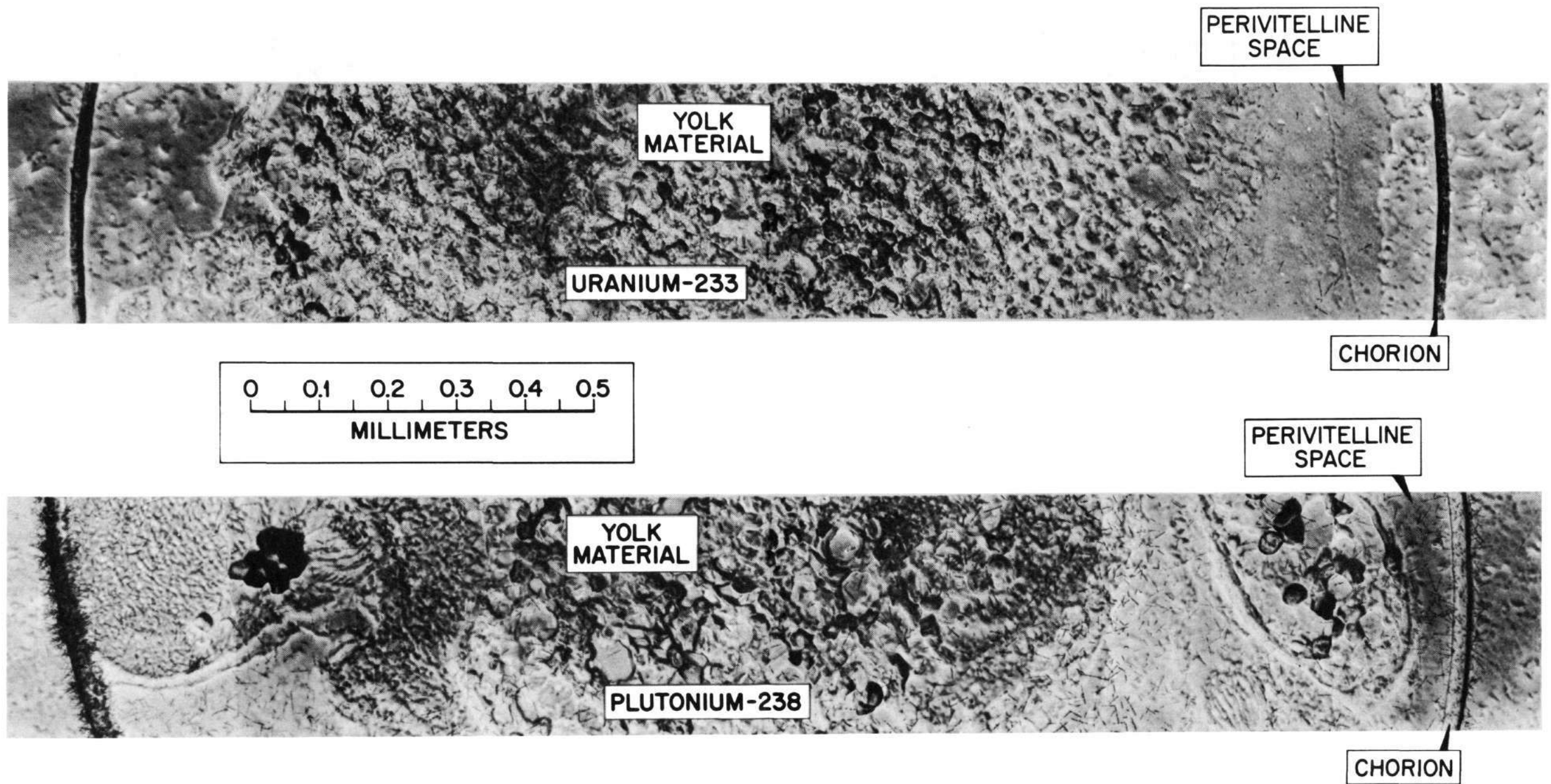


Figure 32. Composite Photomicrograph of Autoradiographs Showing the Distribution of ^{233}U and ^{238}Pu in Carp Eggs

at a magnification of x83 using a green light filter. The original composites were 1.2 m in length. The egg in Figure 32(a) was exposed to ^{233}U for approximately two hours before it was embedded in the acrylamide gel. The egg in Figure 32(b) remained in the ^{238}Pu test solution for approximately 23 hours and organogenesis was in progress when the sample was taken. Autoradiograph exposure periods were 52 days for uranium and 4 days for plutonium.

Toxicity Experiments

Plutonium

Plutonium-238. One objective to the toxicity experiments was to establish an upper limit for the toxicity of plutonium and uranium to fish eggs. This had not been done by previous investigators. Auerbach et al.⁷⁷ reported that no effects were observed on the hatching of carp eggs or the number of abnormal larvae occurring when the eggs were exposed to $5 \times 10^{-2} \mu\text{Ci/ml}$ of Pu(IV)-citrate . Therefore it was decided to begin these experiments at a concentration of ^{238}Pu of approximately $0.1 \mu\text{Ci/ml}$ and increase the concentration until effects were produced.

The toxicity of ^{238}Pu as determined by the number of *C. carpio* eggs that hatched and the survival of larvae is depicted in Figure 33. The data from which this toxicity graph and the following toxicity graphs were taken are contained in Appendix C. All data points represent the mean number of eggs that hatched expressed in percent. Usually three dishes of eggs were exposed at each concentration tested.

Toxicity graphs show 95% confidence limits. These limits were computed by using the normal approximation to the binomial distribution

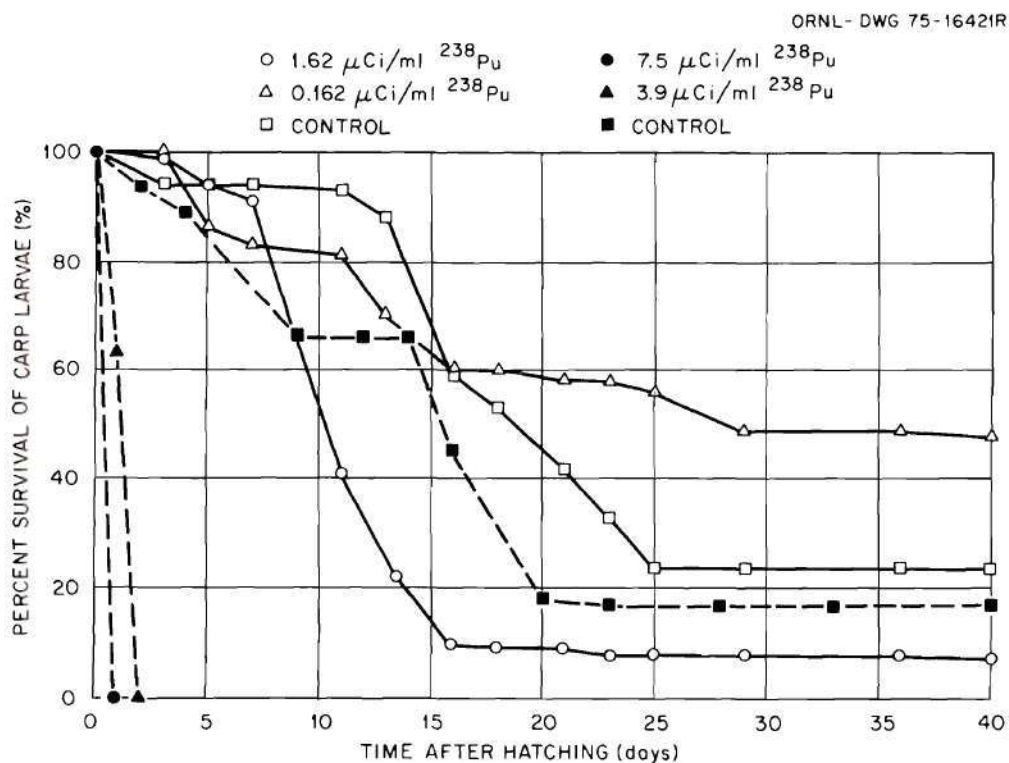
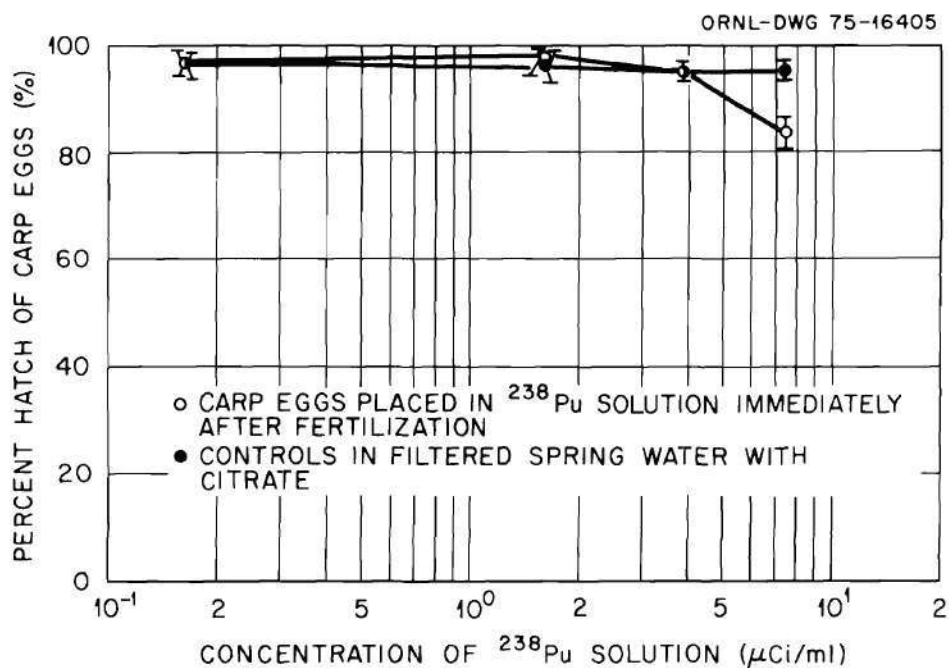


Figure 33. Effect of ^{238}Pu on the Hatching of Carp Eggs and the Survival of Larvae

since the number of eggs tested at each concentration was large. Fisher's Exact Test was used to determine whether there was a significant difference between the number of abnormal larvae produced in control and contaminated solutions. A discussion of this test was reported by Ostle.¹³⁵

A concentration of 7.5 $\mu\text{Ci/ml}$ of ^{238}Pu did not entirely inhibit carp eggs from hatching. However, significant numbers of abnormalities resulted and most of the larvae died within several hours after hatching. At 3.9 $\mu\text{Ci/ml}$ a normal hatching occurred although there was a significantly higher number of abnormalities than in the control group and larvae did not survive more than 48 hours after hatching. Concentrations of 1.6 $\mu\text{Ci/ml}$ and 0.16 $\mu\text{Ci/ml}$ did not affect hatching and no significant effect was observed on the survival of larvae exposed to these levels of activity.

Figure 34 illustrates the results of toxicity experiments and survival of larvae using *P. promelas* eggs as the test organisms. More data points are present in the graph because there were no time limitations imposed by the spawning season as with carp. Again, three separate groups of eggs constituted each point and 95% confidence limits are indicated. Because the fathead egg required seven days to hatch instead of three as with carp, effects were predicted at lower concentrations. Hatching was severely inhibited by 1.3 $\mu\text{Ci/ml}$ of ^{238}Pu and concentrations greater than this. Most of the larvae were abnormal at 0.85 $\mu\text{Ci/ml}$ and many hatched prematurely. Even at 0.26 $\mu\text{Ci/ml}$ there were a significant number of abnormal larvae and approximately one-third

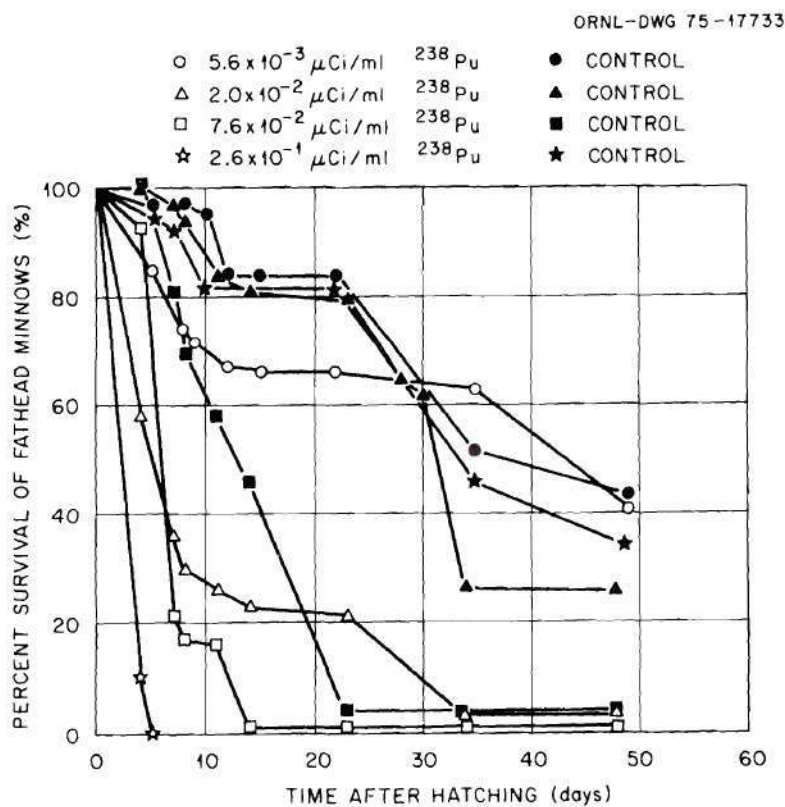
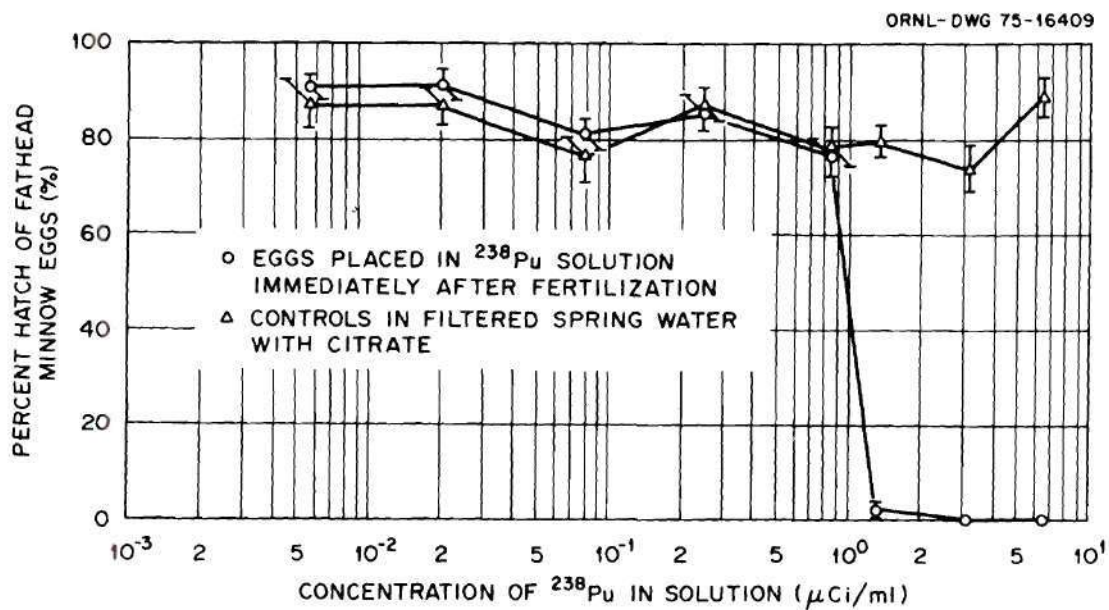


Figure 34. Effect of ^{238}Pu on the Hatching of Fathead Minnow Eggs and the Survival of Larvae

of the larvae hatched 12-24 hours early. The survival of fathead minnow larvae clearly demonstrates that 0.26 $\mu\text{Ci/ml}$ of ^{238}Pu produced no viable fry. A higher mortality rate was also observed during the time the yolk sac was being absorbed for 0.076 $\mu\text{Ci/ml}$ and 0.02 $\mu\text{Ci/ml}$. There were no obvious effects at 0.0056 $\mu\text{Ci/ml}$ on either hatching or survival.

The mass concentration of ^{238}Pu at the maximum activity studied in this experiment, 7.5 $\mu\text{Ci/ml}$, was approximately 0.44 ppm. A maximum upper limit for citrate was $3 \times 10^{-3}\%$.

Plutonium-244. The results of toxicity experiments in which carp eggs and fathead minnow eggs were exposed to ^{244}Pu are shown in Figure 35. The data are presented in Appendix C. At 20 ppm ^{244}Pu , the alpha activity was approximately 2×10^{-2} $\mu\text{Ci/ml}$ and an upper limit for citrate concentration was $3 \times 10^{-2}\%$. Plutonium-238 and citrate were added to control solutions to provide similar radioactivity and citrate concentrations that existed in the test medium.

Twenty parts per million of ^{244}Pu in the test solution prevented both types of eggs from hatching. Carp embryos in 20 ppm ^{244}Pu did not reach the eyed stage before dying and 9 ppm delayed hatching approximately six hours later than controls. Fathead minnow eggs were dead by the fourth day. Control groups for 20 ppm ^{244}Pu had normal hatches. No difference was observed between the survival of control and test larvae.

The limited quantity of ^{244}Pu available for these experiments prohibited determinations for toxicity at concentrations between 10 ppm and 20 ppm. The test solution was recycled in an attempt to obtain

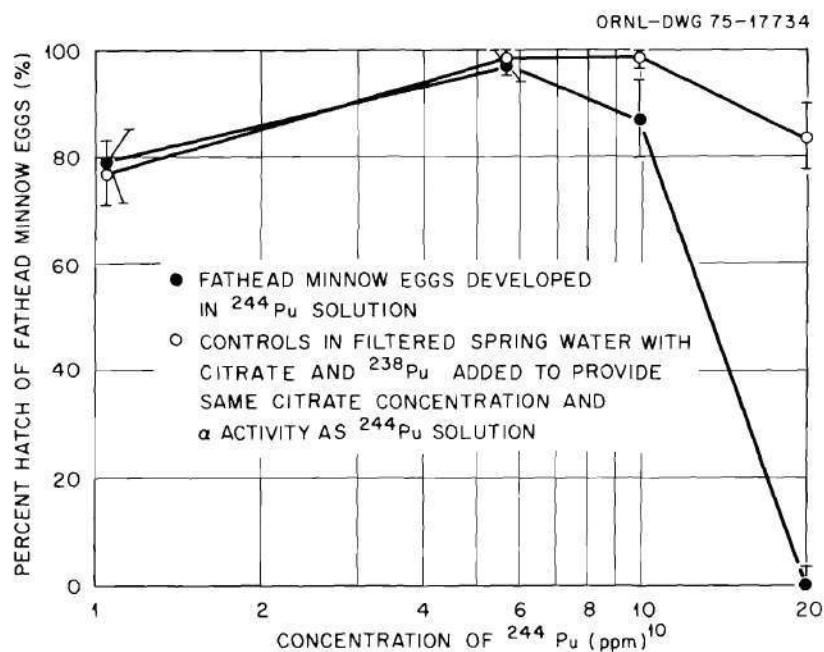
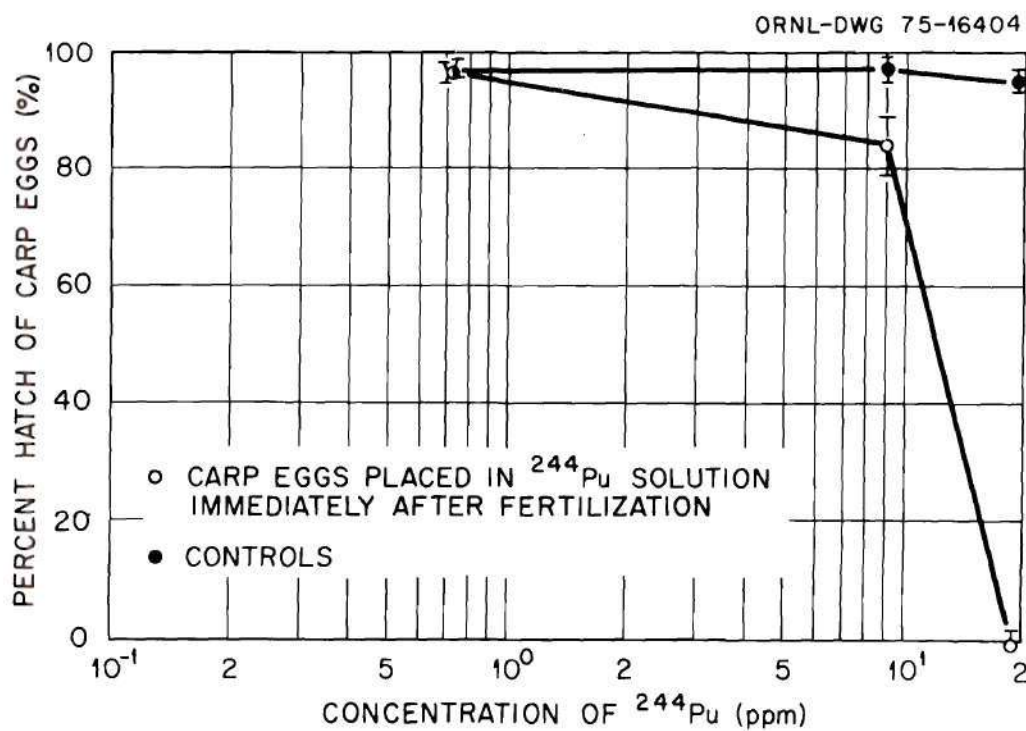


Figure 35. Effect of ^{244}Pu on the Hatching of Carp and Fathead Minnow Eggs

more data at 20 ppm; however, the results of experiments using the recycled mixture were erratic and inconclusive.

Uranium

Uranium-232. The effect of ^{232}U on the hatching of carp eggs and larvae survival is illustrated in Figure 36. Hatching was entirely prevented at 12.5 $\mu\text{Ci/ml}$ and eggs did not live for 48 hours. Embryos exposed to 5.0 $\mu\text{Ci/ml}$ developed fully but were not viable at hatching. A normal hatch was observed at 1.2 $\mu\text{Ci/ml}$ and 0.8 $\mu\text{Ci/ml}$; however, none of the larvae exposed to these levels survived more than 13 days. Also, 12 abnormalities resulted among the 1.2 $\mu\text{Ci/ml}$ group.

Similar data for fathead minnows are presented graphically in Figure 37. None of the eggs hatched that were exposed to concentrations of 0.5 $\mu\text{Ci/ml}$ or greater. Gross abnormalities resulted among larvae at 0.2 $\mu\text{Ci/ml}$ and 5 abnormalities were observed at 0.05 $\mu\text{Ci/ml}$. The survival plot indicated that 0.21 $\mu\text{Ci/ml}$ was lethal, while no effect on survival was noted at exposure levels below this value.

The uranium mass concentration at 12.5 $\mu\text{Ci/ml}$ was approximately 0.6 ppm and the upper limit for citrate in solution was estimated to be $5 \times 10^{-3}\%$.

Uranium-238, 235, 233

The chemical toxicity of uranium to developing carp eggs is demonstrated in Figure 38 along with survival data for test groups. Isotopes employed to obtain each data point are indicated. Citrate was not added to the solutions in which the control groups were developed. These experiments failed to establish a conclusive upper limit

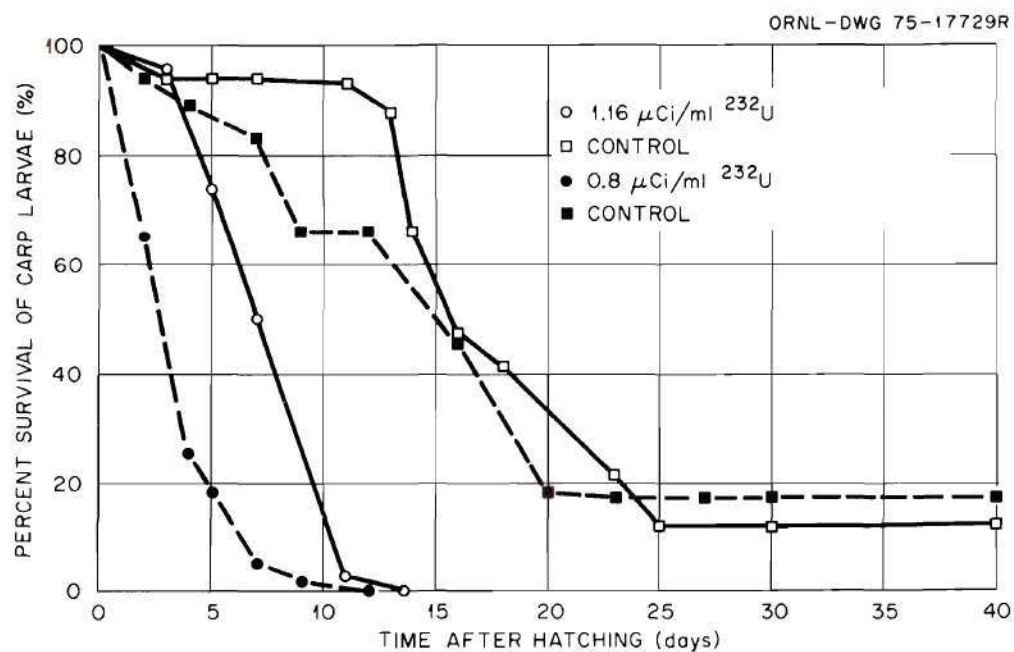
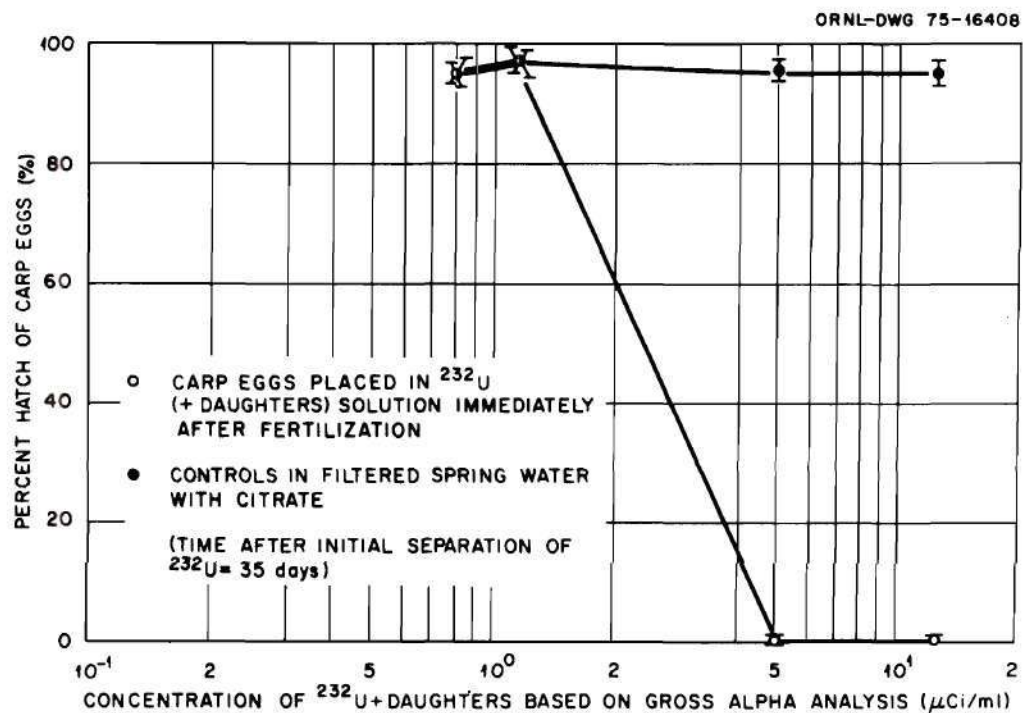


Figure 36. Effect of ^{232}U on the Hatching of Carp Eggs and the Survival of Larvae

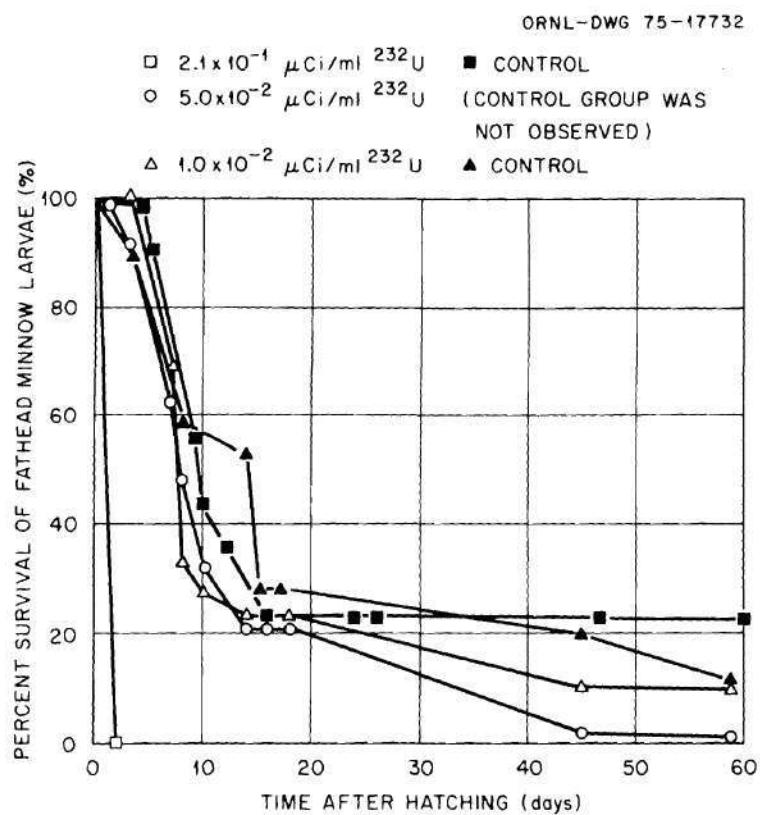
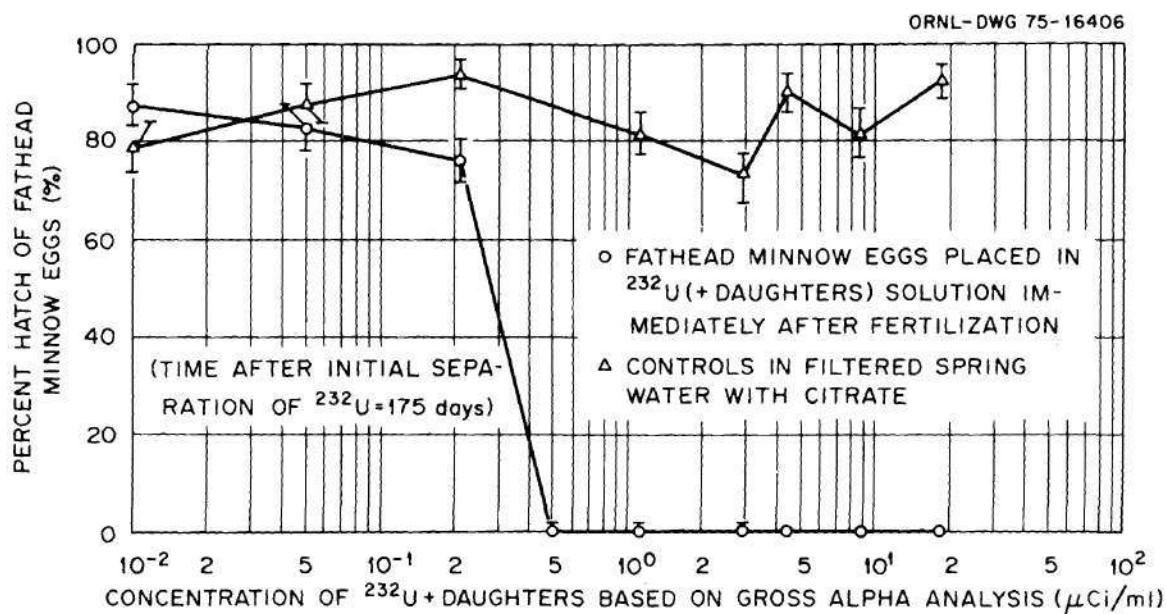


Figure 37. Effect of ^{232}U on the Hatching of Fathead Minnow Eggs and the Survival of Larvae

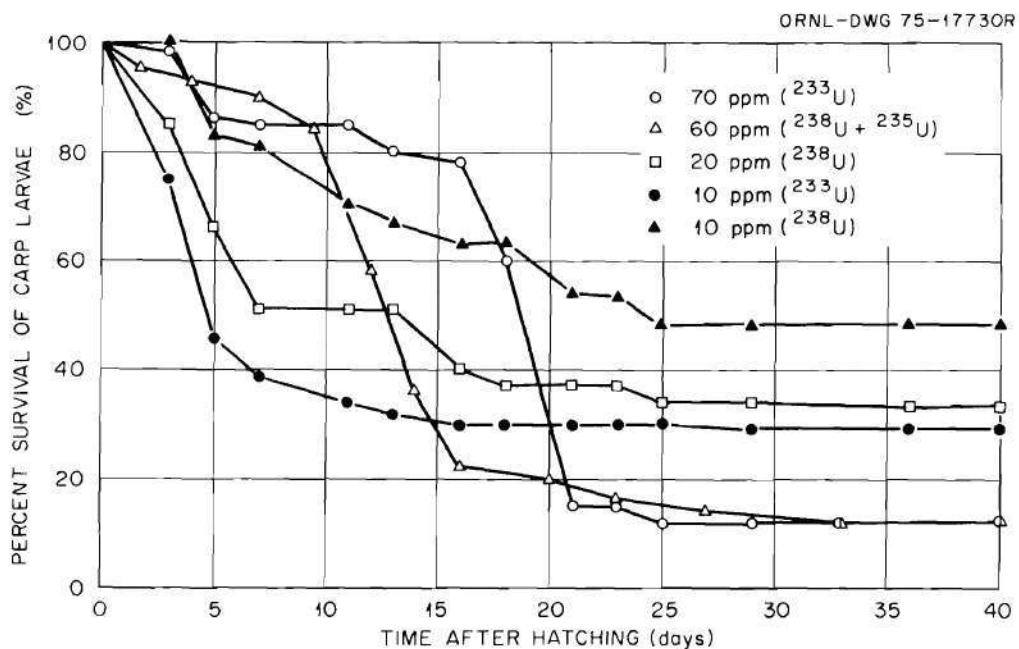
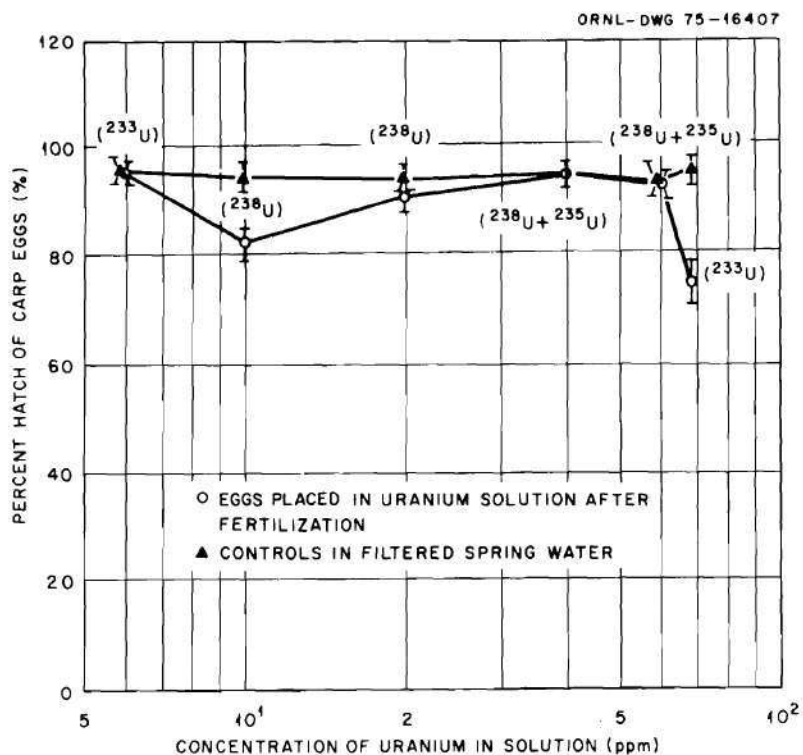


Figure 38. The Effect of ^{233}U , ^{235}U , and ^{238}U on the Hatching of Carp Eggs and the Survival of Larvae

for uranium chemical toxicity. At 70 ppm a 76% hatch resulted in the test group. However, ^{233}U was the isotope used and the outcome of this experiment may have been affected by radiological effects. Only 82% of the eggs hatched that were exposed to 10 ppm ^{238}U while the control group had a 94% hatch. Also, six abnormal larvae were present. The survival of control larvae was not included in the lower graph in Figure 38 in order to minimize the number of lines in the graph. There was, however, no significant difference between the survival of test larvae and control larvae.

Citrate

The toxicity of trisodium citrate, $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$, as determined by the number of carp eggs hatching is shown at the top of Figure 39. A concentration of 0.1% of $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$ caused the eggs to die after 24 hours and a noticeable reduction in hatching occurred at $5 \times 10^{-2}\%$. Although a normal hatch was observed at $10^{-2}\%$, the test group hatched several hours later than controls.

Potassium Fluoride

A toxicity experiment was conducted to establish the effect of potassium fluoride, KF, on carp eggs. The results are depicted in the bottom graph of Figure 39. Hatching was not affected by concentrations of fluoride ion, F^- , of 100 ppm and less. Larvae that hatched at 190 ppm did not survive more than 12 hours. A long-term study of larval survival was not performed.

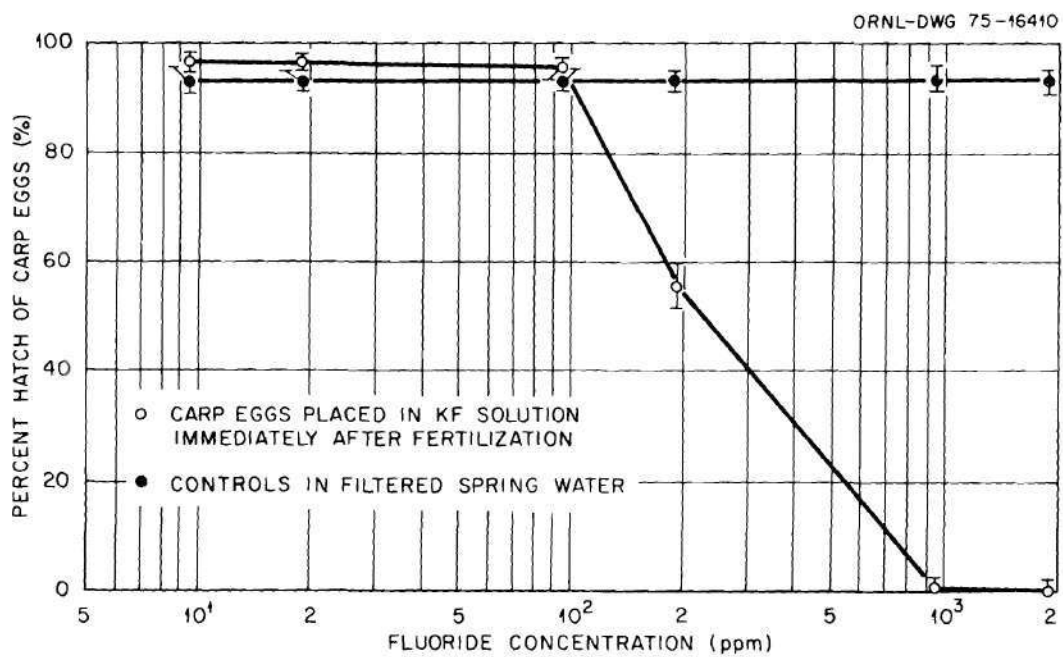
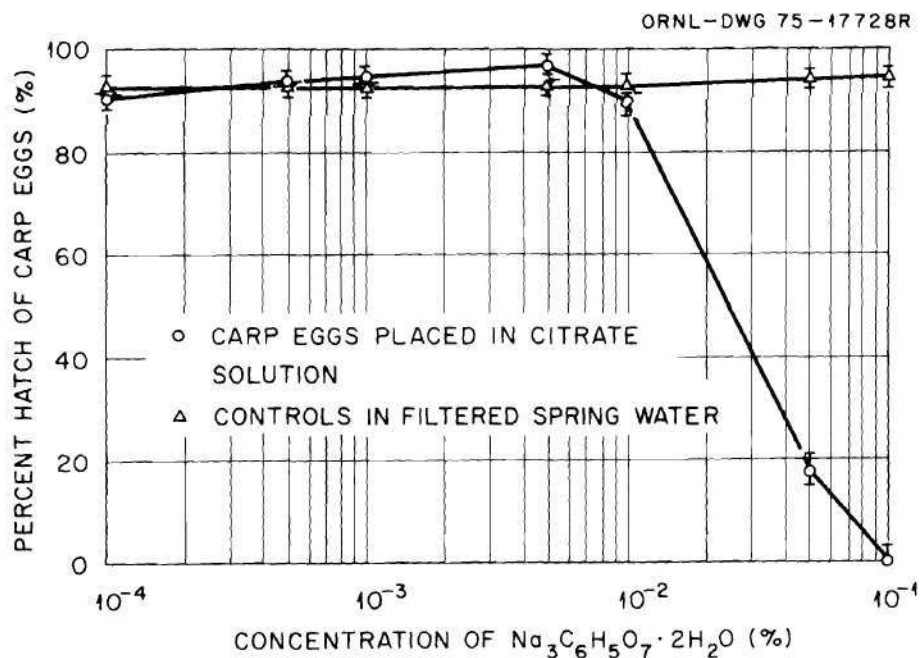


Figure 39. The Effect of Trisodium Citrate and Potassium Fluoride on the Hatching of Carp Eggs

Assessment of the Dose

Introduction

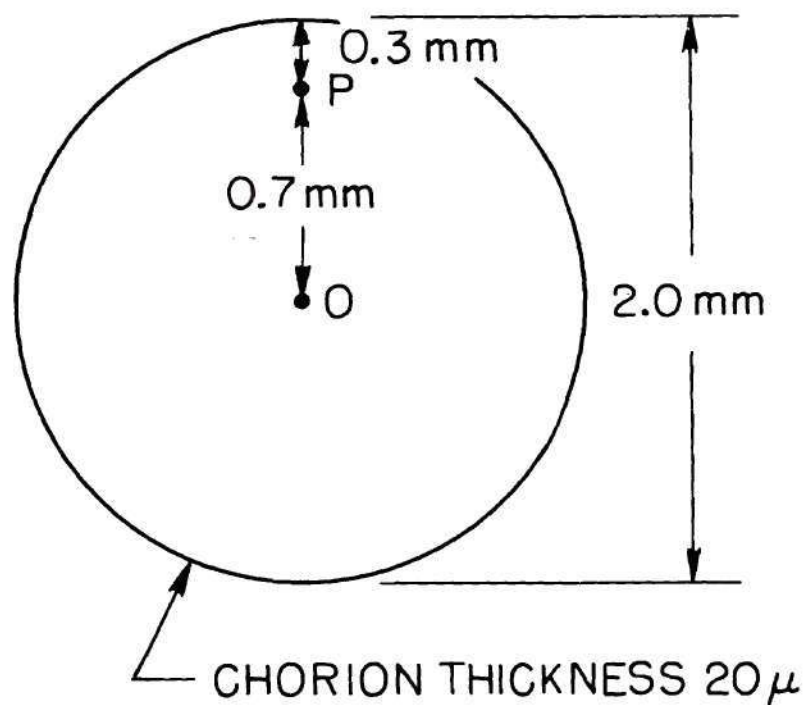
The dosimetry for fish embryos exposed to ^{238}Pu and ^{232}U involved several variables. These included the rate of transport of the radionuclide through the chorion, concentration of the radionuclide in the contents, distribution of the radionuclide inside the egg, geometric characteristics of the egg, length of time it takes for the egg to hatch, and the type of radioactivity being considered. In the literature, there have been no attempts to incorporate all of these variables into dosimetric calculations.

The dimensions for *C. carpio* eggs and *P. promelas* eggs are shown in Figure 40. These dimensions are approximately the same as those assumed by Woodhead³⁹ and Trabalka.⁷⁴ Point P indicates the region of the egg where embryonic development takes place. Embryo diameters and chorion thicknesses were based on measurements made by the author.

The maximum range of an alpha particle from ^{232}U or ^{238}Pu in water is approximately 40 μ . Considering the 20 μ thickness of the chorion and the protection afforded by the perivitelline space surrounding the yolk and blastodisk during early embryonic development, it was assumed that alpha particles originating in the medium surrounding the egg and on the chorion surface did not contribute appreciably to the dose at point P inside the egg.

The geometry was also simplified by assuming that eggs were entirely surrounded by radioactive solution. This leads to an overestimate of the dose since eggs were attached to the bottom of the dish

CYPRINUS CARPIO EGG



PIMEPHALES PROMELAS EGG

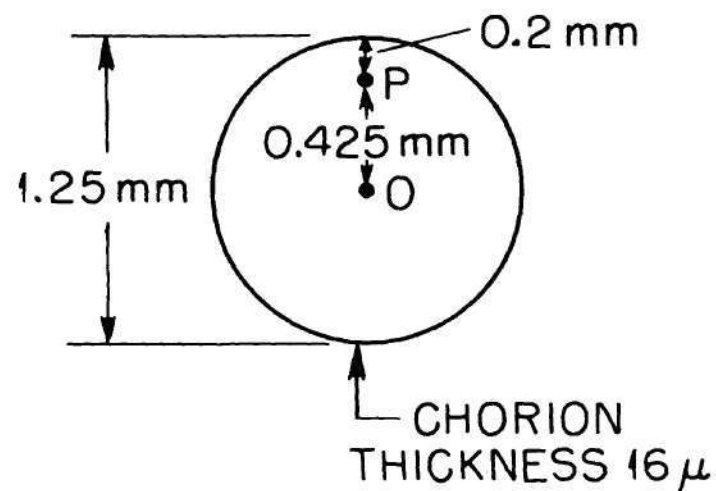


Figure 40. Geometry Used for Estimating Doses

or to the underside of a piece of floating polyethylene. Embryogenesis required 72 hours for *C. carpio* eggs and 168 hours for *P. promelas* eggs.

In the case of ^{238}Pu , the dose rate was a function of the ^{238}Pu concentration in the egg content. Based on autoradiographs, it was assumed that the ^{238}Pu was uniformly distributed throughout the egg volume.

The total dose to the egg from ^{232}U was the result of three types of radiation — alpha, beta, and gamma. The contribution to the total dose from each type of radiation is a function of the degree of equilibrium between ^{232}U and daughters. Since exposure from alpha radiation occurred only when the alpha originated in the egg contents, exposure immediately after eggs were placed in the contaminated solution was from beta and gamma radiation. As the alpha-emitting radionuclides penetrated the chorion, the dose rate to the egg contents from that alpha radioactivity exceeded the dose rate from beta and gamma radioactivity.

In this study, ^{232}U was used approximately 35 days after separation from daughters for *C. carpio* eggs and 180 days after separation from its daughters for *P. promelas* eggs. The concentration of alpha activity in the test solution was determined by gross alpha analysis. Dose rates were calculated for alpha, beta, and gamma radiation for 1 $\mu\text{Ci/ml}$ of alpha radioactivity in the medium surrounding the egg. The effective energy for alpha, beta, and gamma radiation per disintegration of alpha radioactivity as a function of the time after ^{232}U had been separated from its daughters are shown in Figure 41 and Figure 42.

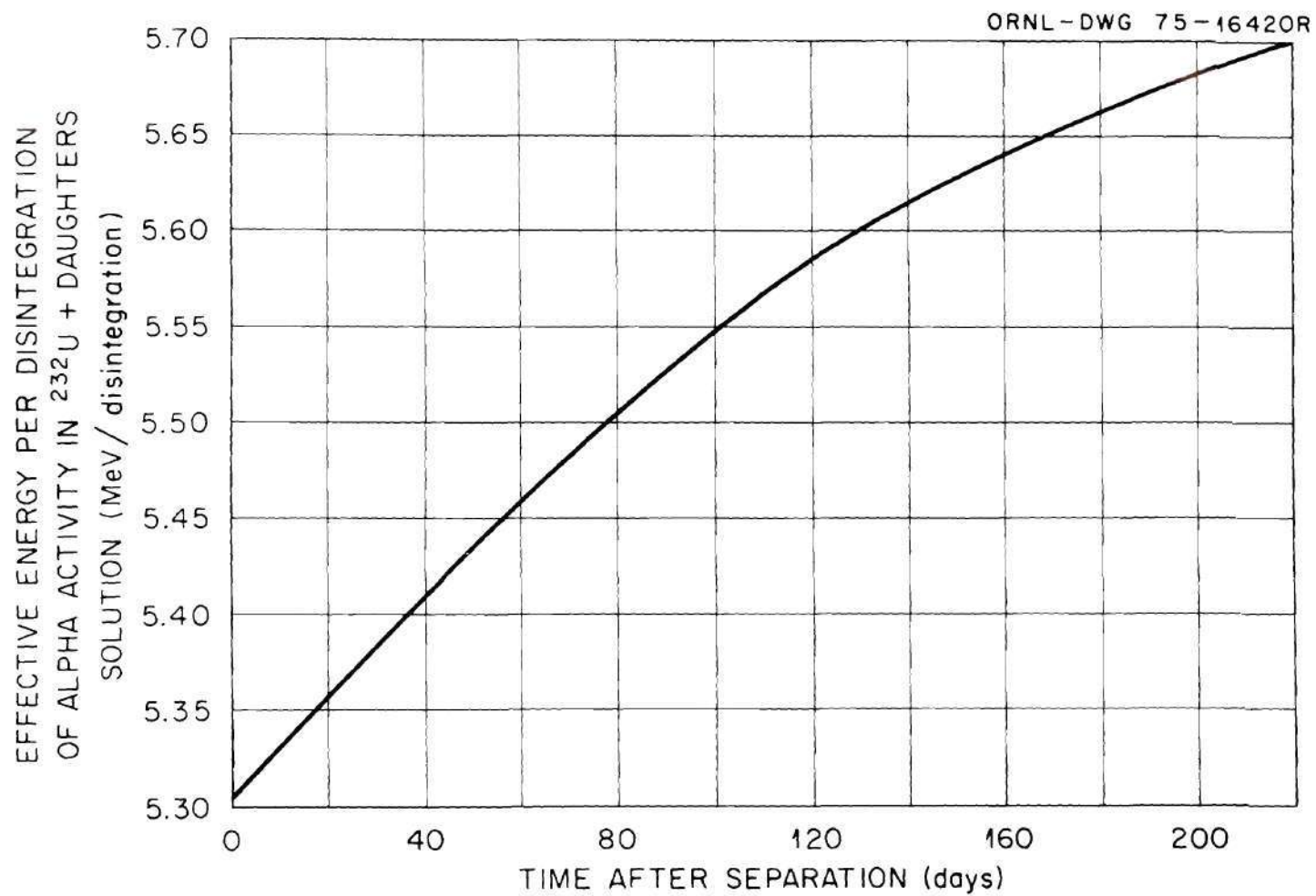


Figure 41. Effective Alpha Energy per Disintegration of Alpha Activity in a Solution of ^{232}U + Daughters

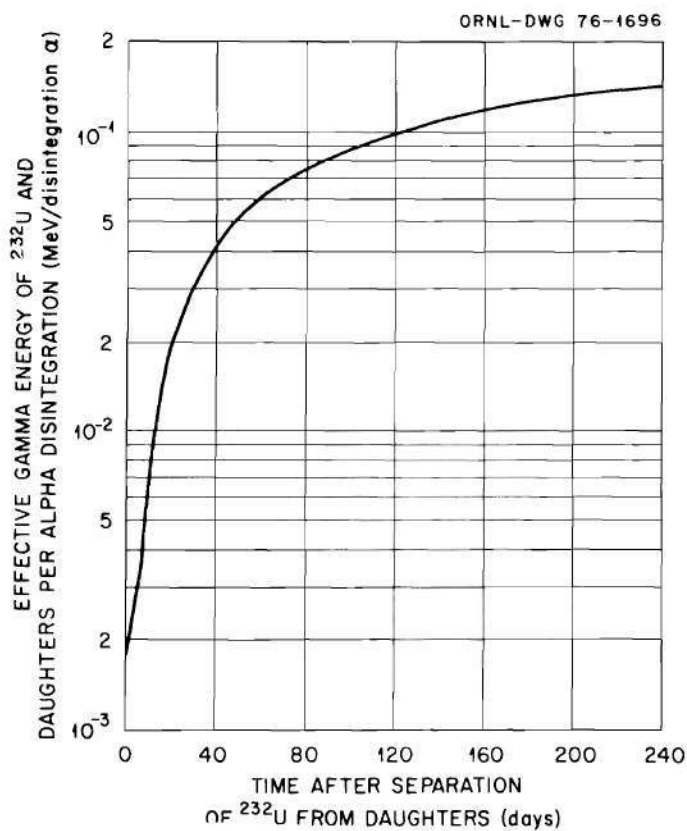
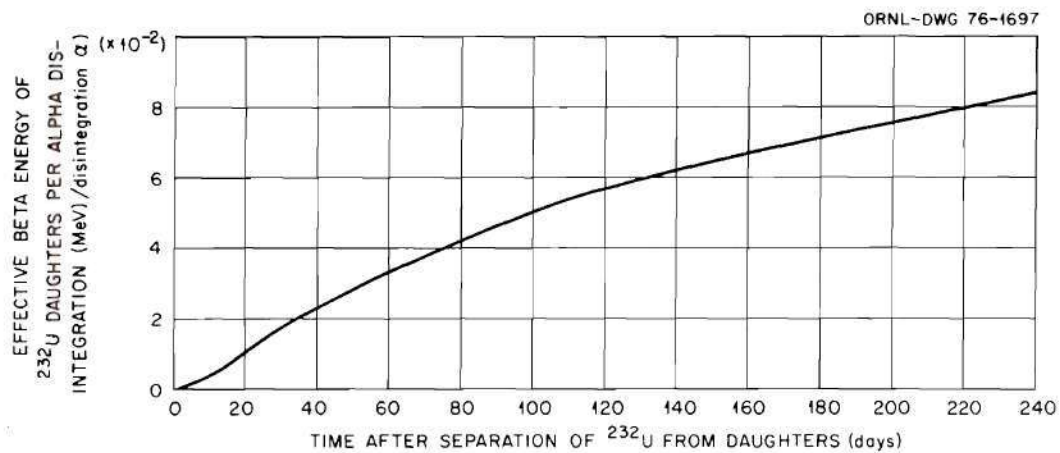


Figure 42. Effective Beta and Gamma Energy per Disintegration of Alpha Activity in a Solution of ^{232}U + Daughters

Dosimetry for Plutonium-238

The dose rate to the embryo from ^{238}Pu was calculated using the following expression:

$$\dot{D}_{\alpha}(t) = 2.13 \epsilon_{\alpha} C \delta(t) \quad \text{rad/hour} \quad (3)$$

where

$\dot{D}_{\alpha}(t)$ = dose rate at time t (rad/hour) to the egg contents from alpha radioactivity,

2.13 = a normalizing constant $\left[\frac{\text{g-rad}}{(\mu\text{Ci-hour})(\text{MeV/dis})} \right]$,

ϵ_{α} = effective absorbed energy (MeV/dis) for alpha radioactivity,

C = concentration ($\mu\text{Ci/ml}$) of alpha radioactivity in the test solution,

$\delta(t)$ = concentration factor (dimensionless) for the egg contents at time t , and

t = time after the eggs were placed into the test solution.

For the purpose of dosimetric calculations, it was assumed that both *C. carpio* eggs and *P. promelas* eggs were placed into the test solution immediately after fertilization. The dose was calculated by integrating Equation 3.

$$D(t) = \int_0^t \dot{D}_{\alpha}(\tau) d\tau \quad \text{rad} \quad (4)$$

$$D(t) = 2.13 \epsilon_{\alpha} C \int_0^t \delta(\tau) d\tau \text{ rad} \quad (5)$$

where

$D_{\alpha}(t)$ = dose (rad) to the egg contents from fertilization to time t , and

τ = time variable of integration (hour).

As previously discussed, the concentration factor for the egg contents for ^{238}Pu as a function of time t was determined to be

$$\delta(t) = \kappa\sqrt{t} \text{ , where } \kappa = 0.44 \quad (1)$$

Substituting this function into Equation 5 and integrating yields the expression for dose from ^{238}Pu .

$$D_{\alpha}(t) = 2.13 \epsilon_{\alpha} C \kappa \int_0^t \sqrt{\tau} d\tau \text{ rad} \quad (6)$$

$$D_{\alpha}(t) = 0.625 \epsilon_{\alpha} C t^{3/2} \text{ rad} \quad (7)$$

The value of ϵ_{α} for ^{238}Pu was 5.49 MeV/dis. If it is assumed that the functions for the concentration of ^{238}Pu in the egg contents for both *C. carpio* eggs and *P. promelas* eggs are identical, then the dose for a unit concentration of alpha activity, $C = 1 \mu\text{Ci/ml}$, is calculated by substituting the egg development period of each species for the time of integration, t . The total homogeneous dose to carp eggs incubated in $1 \mu\text{Ci/ml}$ of ^{238}Pu was estimated to be

$$D_{\alpha}(\text{carp}) = (0.625)(5.49)(1)(72)^{3/2} \text{ rad} \quad (8)$$

$$D_{\alpha}(\text{carp}) = 2096 \text{ rad} \quad (9)$$

The corresponding dose to fathead minnow eggs was calculated by substituting $t = 168$ hours.

$$D_{\alpha}(\text{fathead minnow}) = 7472 \text{ rad} \quad (10)$$

The dose rate in rad/hour as a function of time for eggs exposed to $1 \mu\text{Ci/ml}$ of ^{238}Pu is illustrated in Figure 43.

Dosimetry for Uranium-232

Dose from Alpha Radioactivity. The dose to the fish embryo from alpha radioactivity of ^{232}U and daughters was computed using Equation 3. However, the expression for the concentration factor, $\delta(t)$, was assumed to be independent of time and remained constant throughout development. The concentration factor for uranium was estimated initially to be 0.4 based on the results of quantitative penetration experiments using ^{233}U (see p. 110) and assuming uniform distribution throughout an egg volume of $4.2 \times 10^{-3} \text{ ml}$. However, autoradiographs later revealed that the ^{233}U present in the egg contents was localized primarily in the yolk sac.

For dosimetric calculations, it was assumed that all of the uranium in the contents of the egg was located in the yolk sac even though it was observed that some of the uranium was distributed across the yolk-tissue interface. Therefore the dose computed for the yolk described in

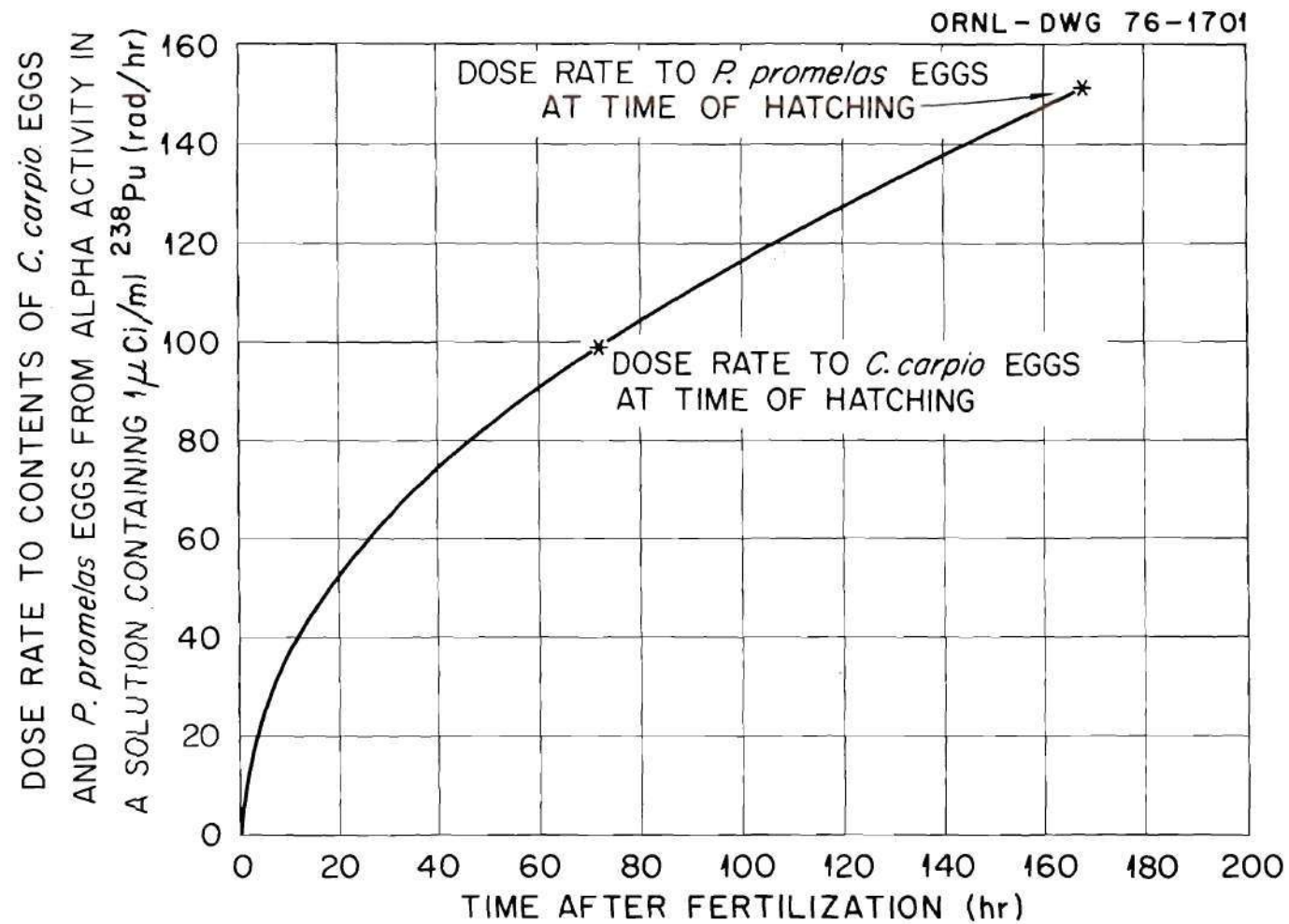


Figure 43. Dose Rate to Carp Eggs and Fathead Minnow Eggs
in a Solution Containing $1.0\mu\text{Ci/ml } ^{238}\text{Pu}$

the following paragraphs would also be approximately equal to the dose at the yolk-tissue interface.

The volume of the yolk sac decreases during embryological development. Based on measurements by this author a mean yolk sac diameter in carp eggs soon after fertilization was found to be 1.25 mm; a corresponding value just prior to hatching was 0.8 mm. The yolk sac diameters for fathead minnows were 0.87 mm at fertilization and 0.43 mm at hatching. For the purpose of dose calculations, mean yolk sac diameters of 1.0 mm for carp and 0.64 mm for fathead minnows were assumed. These diameters resulted in yolk volumes of 5.2×10^{-4} ml and 1.5×10^{-4} ml, respectively.

The concentration factor for uranium in the total egg contents may now be corrected to reflect the localization of uranium in the yolk sac.

$$\delta(\text{yolk sac of carp}) = 0.4 \times \frac{4.2 \times 10^{-3} \text{ ml}}{5.2 \times 10^{-4} \text{ ml}} = 3.23 \quad (11)$$

$$\delta(\text{yolk sac of fathead minnows}) = 0.4 \times \frac{1.0 \times 10^{-3} \text{ ml}}{1.5 \times 10^{-4} \text{ ml}} = 2.67 \quad (12)$$

The dose rate from ^{232}U may now be computed using Equation 3 and a value of $\delta = 3.23$ for carp eggs and 2.67 for fathead minnow eggs.

$$\dot{D}_{\alpha}(\text{carp}) = 6.88 \epsilon_{\alpha} C \text{ rad/hour} \quad (13)$$

$$\dot{D}_{\alpha}(\text{fathead minnows}) = 5.69 \epsilon_{\alpha} C \text{ rad/hour} \quad (14)$$

where

\dot{D}_{α} = dose rate (rad/hour) to the egg yolk from alpha radioactivity,

ϵ_{α} = effective absorbed energy (MeV/dis) for alpha radioactivity, and

C = concentration ($\mu\text{Ci/ml}$) of alpha radioactivity in the test solution.

Total dose in rad to the yolk of an egg exposed to uranium is calculated by integrating Equations 13 and 14 with respect to time.

$$D_{\alpha} = 6.88 \text{ (or } 5.69) \epsilon_{\alpha} C t \text{ rad} \quad (15)$$

The value of ϵ_{α} is taken from Figure 41. For carp experiments which were conducted at 35 days after ^{232}U daughter separation, $\epsilon_{\alpha} = 5.39 \text{ MeV/dis}$. Corresponding ϵ_{α} for fathead minnow experiments was 5.67 MeV/dis at 180 days. The time, t , was 72 hours for carp and 168 hours for fathead minnows. Substituting these data into Equation 15, the dose from a $1 \mu\text{Ci/ml}$ concentration of ^{232}U alpha activity was

$$D_{\alpha}(\text{carp yolk}) = 2670 \text{ rad} \quad (16)$$

$$D_{\alpha}(\text{fathead minnow yolk}) = 5412 \text{ rad} \quad (17)$$

Dose from Beta and Gamma Radioactivity. The dose to fish eggs from beta and gamma radioactivity of ^{232}U daughters was significantly

less than the dose from alpha radioactivity of ^{232}U and daughters. The beta dose was calculated using the procedure outlined by Woodhead.³⁹ In his paper Woodhead divided the dose rate into three components: the dose rate from activity in the egg contents; the dose rate from activity adsorbed on the shell; and the dose rate from activity in the water. The dose was calculated for 1 $\mu\text{Ci/ml}$ of alpha radioactivity from ^{232}U and daughters. The beta particle dose was greater for fathead minnow eggs than for carp eggs because fathead minnow eggs required longer to hatch and because these experiments were performed 180 days after the ^{232}U had been purified. More beta radioactivity was present in solution at 180 days than at 35 days as illustrated in Figure 42. Maximum estimated beta particle doses to carp eggs and fathead minnow eggs from 1 $\mu\text{Ci/ml}$ (alpha radioactivity) of ^{232}U and daughters are listed below.

$$D_{\beta}(\text{carp}) = 3 \text{ rad} \quad (18)$$

$$D_{\beta}(\text{fathead minnow}) = 25 \text{ rad} \quad (19)$$

The most important contribution to the total gamma dose to a fish egg exposed to ^{232}U and daughters comes from ^{208}Tl (2.62 MeV, 100%). The mean attenuation path in water for a photon at this energy is approximately 22 cm. This range was considerably greater than the diameter of the plastic dishes. The gamma dose was estimated using the absorbed fractions for uniformly distributed sources in small unit-density spheres surrounded by scattering medium reported by Ellet and Humes.¹³⁶ Estimated

gamma doses for carp eggs and fathead minnow eggs in a solution of 1 $\mu\text{Ci/ml}$ (alpha radioactivity) of ^{232}U and daughters are listed below.

$$D_{\gamma}(\text{carp}) = 1.4 \times 10^{-3} \text{ rad} \quad (20)$$

$$D_{\gamma}(\text{fathead minnows}) = 1.2 \times 10^{-2} \text{ rad} \quad (21)$$

Summary of the Dose Assessment

Table 10 summarizes the dose to *C. carpio* eggs and *P. promelas* eggs from 1.0 $\mu\text{Ci/ml}$ of ^{238}Pu or ^{232}U as calculated for this experiment.

Table 10. Maximum Estimated Dose to *C. carpio* Eggs and *P. promelas* Eggs Exposed to 1.0 $\mu\text{Ci/ml}$ of ^{238}Pu or ^{232}U During Embryological Development

	Dose(rads)				
	^{238}Pu		^{232}U		
	D_{α}	D_{α}^c	D_{β}	D_{γ}	$D_{\text{Total-}^{232}\text{U}}$
<i>C. carpio</i> ^a	2096	2670	3	0.0014	2673
<i>P. promelas</i> ^b	7472	5420	25	0.012	5445

^aDoses were calculated assuming a 35 day buildup of ^{232}U daughters.

^bDoses were calculated assuming a 180 day buildup of ^{232}U daughters.

^cDose was calculated assuming ^{232}U localized in the yolk sac.

CHAPTER V

DISCUSSION

Behavior of Plutonium and Uranium AssociatedWith the Developing Embryos of FishQuantitative Penetration Experiments

Quantitative experiments led to the conclusion that both plutonium and uranium penetrated the egg chorion and became deposited in the egg volume. The rate of uptake of these elements by fish eggs contrasted significantly. Plutonium accumulation continued throughout development although the rate of accumulation decreased. This indicated that a limited number of sites were available for plutonium transport through the chorion. As the sites became filled, the rate of accumulation decreased. At the same time, however, plutonium migrated through the chorion into the egg contents.

The unusual distribution of the quantitative data showing the activity of ^{233}U in the contents of carp eggs (Figure 28) suggested that uranium was selectively absorbed and then eliminated. However, accumulation by fathead minnow eggs, obtained by analyzing total egg activity, did not yield a similar pattern for uptake. Assuming a uniform distribution of uranium in the egg contents, the mean concentration was 0.42 for carp eggs and 0.38 for fathead minnow eggs. It was concluded from these data that the concentration factor for uranium in the egg contents was a constant value during embryological development. More experimental data

are required to conclusively establish the selective absorption and elimination of uranium before this behavior can be incorporated into dose calculations.

Autoradiographs

Autoradiographs were essential in this study to determine the distribution of plutonium and uranium in the egg contents and to support the data obtained in the quantitative penetration tests. The autoradiographs showed that plutonium was highly localized on the chorion and that subsequent distribution in the egg contents was approximately uniform.

Hot spots of plutonium activity were found on the outside of the membrane. These hot spots did not contribute significantly to the total dose received by the embryo due to the protection afforded by the chorion and perivitelline space. A more in-depth study is necessary to explain the cause of the formation of hot spots. One explanation may be that plutonium attaches to insoluble impurities in the test solution and the impurities adsorb to the egg shell. These particles are too large to penetrate the membrane and remain fixed to the outside. It was observed that the number of hot spots increased proportionately with the length of time the eggs remained in the test solution. This increase supports the theory that hot spots are insoluble impurities.

Another explanation for hot spots was explored. It was thought that the plutonium may continuously accumulate at specific sites on the egg chorion causing them to "grow." This theory was rejected, however, because the size of hot spots did not vary appreciably during the

developmental period of the embryo. Hot spots were similar in size and distribution to the one shown in Figure 31. This suggested that accumulation at specific sites was not occurring. Therefore, hot spots most likely result from plutonium which is attached to insoluble particles in the test solution.

Once inside the egg volume, plutonium and uranium behaved quite differently. Plutonium was uniformly distributed and uranium localized in the yolk. This contrasting biochemical behavior can be explained by the different chemistry of these elements and their distinctive tendency to react with biological materials.

The stability of plutonium complex ion formation decreases in the order: $\text{Pu (IV)} > \text{Pu (III)} > \text{Pu (VI)} > \text{Pu (V)}$. Biological fluids contain complexing entities that tend to stabilize the plutonium (IV) state.¹³⁷ Therefore it was assumed that the plutonium reacting with components of the fish egg remained in the (IV) valence state. Plutonium in the test solution became bound to biological substances in the chorion. As accumulation continued, some plutonium diffused through the chorion and complexed with constituents in the contents. Complexes of plutonium with ligands in biological material have limited mobility. However, nutrients in the embryo, yolk, and cytoplasm are in dynamic equilibrium. The uniform distribution of plutonium in the contents was probably the result of this equilibrium as well as the physical mixing of egg fluids caused by muscular contractions of the embryo during development.

Complex ions in a physiological system compete for any uranium that is present. However, tetravalent actinides like Pu(IV) form stronger associations with donor ligands than do oxygenated actinides of the uranyl type. This property implies that in biological tissue, complexes with the uranyl ion have more mobility than complexes with the plutonium ion. Autoradiographs showed that the uranium in the egg contents was primarily located in the yolk material. A typical teleost egg is approximately 23% protein by mass and essentially all of this protein is in the egg contents.¹⁰³ Protein in the egg contents is concentrated in the yolk sac. The uranyl ion is strongly complexed by protein and the formation of uranium-protein complexes in the yolk sac may explain the buildup of uranium at this site. Protein and other nutrients in the yolk sac are assimilated by the embryo during development. This may account for the general trend reported for the concentration factor of uranium in Figure 28.

The Toxicity of Plutonium and Uranium to the Developing Embryos of Fish

Doses Affecting Hatchability, Survival, and Abnormality Production

Summary of the Doses. Table 11 lists the doses which produced effects on hatching, survival, and abnormalities of carp and fathead minnow eggs. These values were computed using the data listed in Table 10. These data were multiplied by the minimum concentration of test solution at which the effect was observed. For example, hatching of fathead minnow eggs was inhibited by 0.5 $\mu\text{Ci/ml}$ of ^{232}U and higher concentrations. The total dose to fathead minnow eggs incubating in 1.0 $\mu\text{Ci/ml}$

Table 11. Summary of Estimated Doses^a to *C. carpio* and *P. promelas* Eggs from ²³⁸Pu and ²³²U

Radionuclide	Type of Egg	Dose (Rads)		
		Hatching ^b	Survival	Abnormalities (Number Abnormal/ Sample Size)
²³⁸ Pu	<i>C. carpio</i>	1.57×10^4 (8.17×10^3)	8.19×10^3	4.27×10^3 (5/377)
	<i>P. promelas</i>	9.71×10^3 (5.60×10^3)	1.94×10^3	5.68×10^2 (15/454)
²³² U ^c	<i>C. carpio</i>	1.34×10^4 (3.21×10^3)	2.14×10^3	3.21×10^3 (12/421)
	<i>P. promelas</i>	2.72×10^3 (1.20×10^3)	1.20×10^3	2.71×10^2 (5/238)

^aExcept where noted, dose is for the concentration that first produced a significant effect on hatching, survival, and abnormalities.

^bNumber in parentheses is the dose at which little or no effect was observed on hatching and may be considered as an estimate of the threshold dose to affect hatching.

^cUranium-232 dose is calculated assuming all of the ²³²U is localized in the yolk.

of ^{232}U was estimated to be 5445 rads (Table 10). Therefore the dose which prevented egg hatching is calculated as shown below.

$$0.5 \frac{\mu\text{Ci}}{\text{ml}} \times 5445 \frac{\text{rads}}{\mu\text{Ci/ml}} = 2720 \text{ rads} \quad (20)$$

Interpretation of the data in Table 11 requires further clarification. First, the doses to eggs from ^{232}U are assumed to be the doses to the egg yolk only, while the doses from ^{238}Pu may be applied to all of the constituents of the egg. Second, the doses listed do not necessarily represent levels of minimum sensitivity. Many data points are needed on each toxicity curve to establish the concentration that first produces an effect on abnormalities, survival, or hatching. Of particular importance is the need to establish an LD_{50} value which would permit a common basis for comparing radiosensitivity.

Analysis of the Doses. The doses in Table 11 are listed in units of rads. These doses would be significantly greater in units of the dose equivalent, rem. For alpha radioactivity this is calculated by multiplying the dose in rads by the quality factor for alphas which is 10.¹⁰⁴ Therefore, doses in Table 11 in units of rem would be a factor of 10 greater than the doses listed in units of rads. These high dose values suggest that fish eggs are relatively insensitive to the effects of alpha radiation. However, fish eggs were protected from exposure by alpha radioactivity until the alpha-emitting radionuclide has penetrated the chorion and has been distributed at critical sites in the embryo. Therefore, eggs were protected from exposure during the most sensitive period of development, immediately after

fertilization. As the plutonium or uranium penetrated the chorion, the dose rate increased, but the radioresistance of the embryo also increased. For penetrating radiation such as high energy beta or gamma, energy is deposited throughout the egg volume as soon as exposure begins. Therefore, it would not be correct to report doses calculated in this study in units of the dose equivalent without a more in-depth study of the transport through the chorion and distribution of the plutonium and uranium in the egg contents.

The data in Table 11 also suggest that fathead minnow eggs were more sensitive to both ^{238}Pu and ^{232}U than carp eggs. However, although the embryologic characteristics of these two eggs are similar, they may not have the same radiosensitivity. One reason for this is that the fathead minnow is a nest builder, and the male protects the eggs during development. Carp do not protect their eggs during development and hence carp eggs may be more resistant to biological damage than fathead minnow eggs.

In general, effects were observed to occur at lower total doses for ^{232}U than for ^{238}Pu . This greater radiotoxicity for ^{232}U may be attributed to one of the following factors or a synergism between these factors.

(1) The total dose from ^{232}U was calculated assuming the concentration factor for uranium in the egg contents remained constant throughout development. Consequently, the dose rate from ^{232}U was a constant value. However, the concentration factor for uranium was determined using ^{233}U which has a much lower specific activity than

^{232}U . Therefore, it is possible that the concentration factor determined using ^{233}U may have been less than the concentration factor for ^{232}U in toxicity experiments.

The concentration factor for plutonium, on the other hand, was a function of the time the egg remained in the test solution. The dose rate from ^{238}Pu was low during the early stages of development and increased to a maximum value at hatching. Therefore, embryos received a greater dose from ^{232}U than from ^{238}Pu while they were in the most sensitive periods of embryogenesis.

(2) The mobility of uranium caused a rapid buildup of this element in the egg yolk where it was possibly bound as uranium-protein complexes. Casarett,¹³⁸ in reviewing the effects of radiation on proteins, discussed several mechanisms that lead to the loss of function of proteins. These mechanisms included radiation-induced changes in a critical side chain attached to the protein molecule and breakage of hydrogen or disulfide bonds which maintain the protein structure. In addition to direct damage to protein molecules, radiolysis of aqueous solutions leads to the formation of hydrogen atoms, hydroxyl radicals, and molecular hydrogen and hydrogen peroxide.¹³⁹ These reactants may be chemically toxic to the developing embryo. Future work is needed to determine more precisely the radiobiological effects of ^{232}U during embryogenesis of the fish egg.

(3) The radiotoxic effect of ^{232}U daughters may have contributed significantly to the overall toxicity of ^{232}U . The biochemical behavior of each daughter, including transport through the chorion and complex

ion formation with constituents in the egg contents, was likely very different from uranium. In addition to biochemical differences, several ^{232}U daughters emit alpha particles of unusually high energies. This high energy ionizing radiation may have produced more biological damage than lower energy alpha radioactivity.

(4) Eggs incubating in ^{232}U received exposure from three types of radiation - alpha, beta, and gamma. Alpha radioactivity did not contribute significantly to the absorbed dose until after the ^{232}U penetrated the chorion. Beta and gamma radioactivity deposited energy in the egg contents immediately after the egg was placed in the ^{232}U solution, during the most sensitive stage of embryological development. However, due to the low dose rates of beta and gamma radioactivity in the ^{232}U solution, the importance of this factor was considered to be minimal.

One important physiological effect that was noticed at high concentrations of ^{238}Pu activity was premature embryo hatching. This effect was most likely due to the buildup of ^{238}Pu on the egg chorion which resulted in physical damage by intense alpha radioactivity. As embryogenesis proceeded and motility began, muscular contractions by the embryo created pressure on the inside of the weakened shell and eventually ruptured it prematurely.

Comparison of the Doses to Literature Data. Most of the literature reviewed in Chapter I which dealt with experimental data on chronic exposure failed to incorporate estimated doses into the findings. Usually only the concentrations of radionuclides in solution were

reported. This lack of information, along with the limited amount of experimental data on chronic exposure available, made it difficult to compare the doses calculated in this study to those reported in other experiments. Also, caution had to be exercised when comparing acutely administered doses to those delivered chronically.

Brown and Templeton⁴¹ and Templeton⁴² chronically exposed the eggs of plaice (*Pleuronectes platessa*) to exposures up to 500 R but did not observe a significant effect on the number of abnormal larvae produced. Bonham and Donaldson irradiated the eggs of chinook salmon (*Oncorhynchus tshawytscha*) during development and markedly inhibited gonadal development at a total dose of approximately 700 rads.

Frank⁶⁰ demonstrated that very large doses of radiation were required to significantly inhibit hatching and to produce abnormalities when acute doses were administered during the late stages of embryologic development. A total dose of 16,000 rads at 24 hours after fertilization did not interfere with hatching although 4,000 rads or more caused the formation of gross abnormalities. Hyodo-Taguchi⁶⁵ also reported that high doses of neutrons were needed to affect the hatching of *Oryzias latipes* embryos when exposure occurred during late embryogenesis.

Recent experiments by Trabalka¹⁴⁰ found that chronic exposure of carp eggs in a solution having 5 mCi/ml of tritium did not prevent hatching although significant numbers of gross abnormalities resulted. The total dose to the embryos during development was estimated to be approximately 4.4×10^3 rads in 72 hours.

Therefore, although doses affecting hatchability, survival, and abnormality production computed in this experiment could not be compared directly to values determined by other investigators, doses were well within the range of values reported in the literature. Future experiments evaluating the toxicity of radionuclides to developing embryos in aquatic environments should incorporate quantitative factors for bioaccumulation and activity distribution into dose estimates.

The Chemical Toxicity of Plutonium and Uranium

A mass concentration of 20 ppm of ^{244}Pu in solution prevented both carp eggs and fathead minnow eggs from hatching. The control groups were developed in a solution having the same citrate concentration, $3.0 \times 10^{-2}\%$, and activity, $2.0 \times 10^{-2} \mu\text{Ci/ml}$, as the ^{244}Pu . An activity of ^{238}Pu of $2.0 \times 10^{-2} \mu\text{Ci/ml}$ corresponds to a dose of 42 rads to carp eggs and 149 rads to fathead minnow eggs. Experiments at 20 ppm were conducted with one or two dishes of eggs because only a limited amount of the ^{244}Pu was available for this study. Attempts to recycle the test solution following filtration were not successful. According to the results of citrate toxicity on developing eggs shown in Figure 39, a citrate concentration of $3.0 \times 10^{-2}\%$ was on the shoulder of the toxicity curve. Therefore, the potential effect of citrate toxicity rather than plutonium toxicity cannot be entirely ruled out. It was concluded, however, based on the absence of effects from ^{238}Pu at 42 rads and 149 rads that hatching was most likely prevented at 20 ppm ^{244}Pu because of the chemical toxicity of either plutonium or citrate.

The potential chemical toxicity of plutonium at 20 ppm is significant. The most abundant plutonium isotope by mass in the nuclear fuel cycle and in the environment is ^{239}Pu . This isotope has a half-life of 2.44×10^4 years, a specific activity of 6.2×10^{-2} Ci/g, and decays by alpha emission. An aqueous solution of 20 ppm of ^{239}Pu would have an activity concentration of approximately 1.2 $\mu\text{Ci/ml}$. This concentration corresponds to a total dose of 2600 rads to carp embryos and 9270 rads to fathead minnow embryos. A dose of 2600 rads would not prevent carp eggs from hatching and is also less than the dose at which abnormalities were observed (4270 rads). Therefore carp eggs exposed to 1.2 $\mu\text{Ci/ml}$ of ^{239}Pu may be affected by the chemical toxicity of the plutonium rather than the radiotoxicity. Fathead minnows on the other hand would more likely be affected by the radiation although the high concentration of plutonium mass present in 1.2 $\mu\text{Ci/ml}$ of ^{239}Pu may cause a synergistic effect between chemical and radiological toxicity. Consequently, the toxicity of ^{239}Pu to aquatic biota which possess a high degree of radio-resistance may be based on chemical rather than radiological factors.

An upper limit for the chemical toxicity of uranium was not conclusively established. Hatching and survival of carp eggs were not affected by 60 ppm of uranium having an activity concentration less than 2.0×10^{-5} $\mu\text{Ci/ml}$. The results of these experiments indicated that fish eggs were relatively insensitive to the chemical toxicity of uranium.

Carp eggs were also very insensitive to the toxic effect of fluorides. Concentrations less than 100 ppm F^- did not affect hatching. Since these experiments were not exhaustive, this should not be interpreted

to represent an upper limit for fluoride toxicity. More extensive research is needed which focuses actively on fluoride toxicity in an aquatic environment before an upper limit can be established.

Doses to Fish Eggs from Plutonium and Uranium in Natural Waters

Measured values of actual plutonium concentrations in natural waters have varied significantly from one location to another. Several of these values taken from the literature are listed in Table 12. The highest concentrations, 1×10^{-9} $\mu\text{Ci/ml}$, were in ocean waters near the nuclear reprocessing plant at Windscale, England and in a holding pond at the Rocky Flats, Colorado plutonium facility. More typical values ranged between 1×10^{-12} $\mu\text{Ci/ml}$ in Lake Michigan to 4×10^{-15} $\mu\text{Ci/ml}$ in the North Sea. The most significant source of plutonium entry into the aquatic environment today is from controlled releases at nuclear facilities and uncontrolled releases at waste burial grounds.^{11,12} Therefore higher concentrations of plutonium are expected to occur in surface waters around these sites.

Table 13 lists the doses estimated on the basis of the present work for several species of fish eggs developing in natural waters having plutonium concentrations given in Table 12. The total dose in rads was calculated using Equation 7. All of the doses are below levels at which effects on hatchability, survival, or abnormality production would be expected to occur.

There have been essentially no releases of ^{232}U to the environment up to this time because the thorium fuel cycle has not yet been fully developed. Therefore, it was not possible to estimate doses to

Table 12. Concentration of Plutonium Found in Natural Waters

<u>Location</u>	<u>Plutonium Concentration</u> <u>$\mu\text{Ci/ml}$</u>	<u>Year</u>	<u>Reference</u>
Windscale	1×10^{-9}	1975	11
White Oak Lake, ORNL	9×10^{-11}	1974	141
Pond, Hanford	8×10^{-13}	1974	142
Eniwetok, Lagoon	4×10^{-11}	1972	143
Lake Michigan	1×10^{-12}	1971	144

Table 13. Estimated Doses to Fish Embryos Exposed to Plutonium in Natural Waters

Type of Embryo	Length of Embryogenesis ^a (days)	Dose to the Embryo from Plutonium Radioactivity (rads)				
		Windscale	White Oak Lake, ORNL	Pond, Hanford	Eniwetok Lagoon	Lake Michigan
Carp (<i>Cyprinus carpio</i>)	3	2.1×10^{-6}	1.9×10^{-7}	1.7×10^{-9}	8.4×10^{-8}	2.1×10^{-9}
Fathead Minnow (<i>Pimephales promelas</i>)	7	7.5×10^{-6}	6.7×10^{-7}	6.0×10^{-9}	3.0×10^{-7}	7.5×10^{-9}
Brown Trout (<i>Salmo trutta</i>)	58 (41)	1.8×10^{-4}	1.6×10^{-5}	1.7×10^{-6}	7.1×10^{-6}	1.8×10^{-7}
Largemouth Bass (<i>Micropterus salmoides</i>)	3 (97)	2.1×10^{-6}	1.9×10^{-7}	1.7×10^{-9}	8.4×10^{-8}	2.1×10^{-9}
Plaice (<i>Pleuronectes platessa</i>)	18 (41)	3.1×10^{-5}	2.8×10^{-6}	2.5×10^{-8}	1.2×10^{-6}	3.1×10^{-8}
Mummichog (<i>Fundulus heteroclitus</i>)	11 (97)	1.5×10^{-5}	1.3×10^{-6}	1.2×10^{-8}	5.9×10^{-7}	1.5×10^{-8}

^aReference from which the length of embryogenesis was obtained is indicated in parentheses.

fish eggs developing in natural waters containing low concentrations of ^{232}U . However, for comparative purposes, the dose was calculated for carp and fathead minnow eggs in a solution of ^{232}U at $8.0 \times 10^{-6} \mu\text{Ci/ml}$, the MPC_w (168 hours/week) established by the ICRP.¹⁰⁴ This concentration would deliver a dose of 2.1×10^{-2} rads to carp embryos and 5.3×10^{-2} rads to fathead minnow embryos. Again, these values are below levels at which observable effects would be expected to occur.

In summary, levels of plutonium and high specific activity uranium currently found in natural waters do not impart a significant radiological dose to developing fish embryos.

An Evaluation of Fish Eggs as a Toxicity Test System

The advantages and disadvantages associated with the use of fish eggs as a toxicity test system were discussed in Chapter II. In view of the findings in this study, additional elaboration of this test system is possible.

Generally high levels of radiological and chemical toxicants are required to affect the hatching of fish eggs or to significantly reduce the survival of larvae. Tests in which hatchability is the only criterion for biological damage are of limited value. The next step in sensitivity is obtained by observing the survival of larvae at least through the post-larval stage up to the time the yolk sac is absorbed. The production of abnormal larvae adds yet another degree of sensitivity although this effect is more difficult to score, particularly when the work is being performed under the physical restraints imposed by a glove box.

It is likely that the sensitivity would be increased further if fecundity and abnormality production in offspring of fish exposed during embryogenesis could be monitored. The fathead minnow test system developed in this study would be ideally suited for this kind of experiment because this fish is relatively small and could be maintained in laboratory aquaria. Also, breeders reach sexual maturity in approximately four months.

Experiments in which eggs are exposed during development to contaminants dissolved in the solution should incorporate quantitative measurements of accumulation of the contaminant as well as distribution in the egg contents. These data are particularly important when investigating the effects of alpha and beta radioactivity.

CHAPTER VI

CONCLUSIONS

Research to assess the total impact of plutonium and uranium in terrestrial and aquatic ecosystems must proceed concomitant with the development of the nuclear industry. Plutonium, because of its complex chemical behavior and potentially high toxicity to man, has been studied extensively by scientists since it was first discovered in 1941. The potential use of ^{233}U as a fissile material and the presence of impurity ^{232}U in this fuel require that additional research be conducted on high specific activity uranium. This study has focused on the toxicity of uranium and plutonium to one type of aquatic biota, the fish egg.

The literature review established that considerable data were available on the effects that ionizing radiation has on the developing embryos of fish. However, essentially no information was reported on the effects of alpha-emitting radionuclides, specifically plutonium and uranium.

A toxicity test system employing fertilized eggs of carp, *Cyprinus carpio* L., and fathead minnows, *Pimephales promelas* R., was used to determine the radiological and chemical toxicity of plutonium and uranium. Carp eggs were obtained following a laboratory spawning technique reported in the literature. A plentiful supply of fathead minnow eggs was provided by placing breeders in laboratory aquaria equipped with artificial shelters.

Plutonium penetrated the chorion and was accumulated by the contents of the embryo during development. The concentration factor in carp eggs was approximately 3.0 at the time the eggs hatched. The concentration factor for uranium, assuming uniform distribution in the egg, remained constant at a mean value of 0.4.

Autoradiographs indicated that plutonium was uniformly distributed throughout the cytoplasm, embryo, and yolk sac. Approximately 70% of the plutonium activity associated with the total egg was on the chorion. Although hot spots of plutonium activity were present on the outside of the egg shell, they did not contribute significantly to the absorbed dose in the contents of the egg. Autoradiographs of egg sections that were exposed to uranium revealed that this element was primarily localized in the yolk sac.

Relatively high doses and hence high concentrations of ^{238}Pu and ^{232}U , were needed to affect the hatchability of either carp eggs or fathead minnow eggs. A more sensitive test for toxicity, however, was the production of abnormal larvae. A significantly higher number of abnormalities were produced at doses ranging between 4.27×10^3 rads for carp eggs exposed to ^{238}Pu and 3.28×10^2 rads for fathead minnows exposed to ^{232}U . In general, effects were observed at lower total doses for ^{232}U than for ^{238}Pu .

A chemical toxic effect from plutonium was observed by the failure of both carp eggs and fathead minnow eggs to hatch in a solution having a mass concentration of 20 ppm and an activity level of 2.0×10^{-2} $\mu\text{Ci/ml}$. Chemical effects from the presence of citrate could not be

entirely ruled out; however, the potential chemical toxicity of plutonium to a large part of the aquatic biota may be more important than the radiological toxicity. Further study is definitely needed in this area and experiments should be expanded to include mammalian systems. A chemical toxic effect from low specific activity uranium was not observed.

Doses to fish embryos in natural waters contaminated with plutonium or uranium were calculated using the accumulation, distribution, and dosimetry developed in this study. Even in waters where levels are three orders of magnitude greater than mean concentrations, doses would likely not exceed 1.8×10^{-4} rads for an egg that requires 58 days to hatch. This dose is well below the level at which effects would be expected to occur.

The metabolic and environmental behavior of high specific activity uranium, in particular ^{232}U , require further investigation. Very little information is available in the literature on ^{232}U and its daughters even though this isotope of uranium may be present in significant quantities in the thorium fuel cycle.

This work has established the suitability of fish eggs as an indicator for toxic effects from dissolved alpha emitters. However, fish eggs are not a very radiosensitive system, and further work may be needed to relate effects in fish eggs to biological effects in other organisms.

APPENDIX A
GLOVE BOX OPERATIONAL CERTIFICATION

March 18, 1976

To: R. E. Leuze

From: F. A. Kappelmann

Subject: Personnel Certified for Glove Box Operation, Building 3508

During 1975 the following personnel were certified to perform glove box operations in Building 3508.

Chemical Technology Division —

G. D. Davis
R. L. Hickey
F. A. Kappelmann
L. E. Morse

Chemistry Division —

H. A. Friedman

Environmental Sciences Division —

Marilyn Franks
M. H. Shanks
Fred Sweeton
J. E. Till

The above personnel will be requested to attend the annual meeting for recertification to be held in April, 1976. The reason I picked April is because the Operations Manual and Radiation Safety Procedures for Building 3508 should be completed or nearly completed at that time.



F. A. Kappelmann

FAK:jp

APPENDIX B

ANALYTICAL DATA FOR QUANTITATIVE PENETRATION STUDIES

Table 14. Analytical Data for ^{238}Pu
Quantitative Penetration Studies

Sample Number	Time After Fertilization That Eggs Were Placed in the Test Solution (hours)	Time Eggs Remained in the Test Solution (hours)	Type of Sample C=Contents M=Membrane T=Total Egg	Sample Size
1-1	0	2.4	C	50
1-2	0	2.4	M	50
1-3	0	2.4	T	79
2-1	0	8.8	C	53
2-2	0	8.8	M	53
2-3	0	8.8	T	100
3-1	0	23.0	C	48
3-2	0	23.0	M	48
3-3	0	23.0	T	50
4-1	0	46.0	C	31
4-2	0	46.0	M	31
4-3	0	46.0	T	72
5-1	2	1.8	C	53
5-2	2	1.8	M	53
5-3	2	1.8	T	81
6-1	2	7.6	C	50
6-2	2	7.6	M	50
6-3	2	7.6	T	63
7-1	2	22.0	C	43
7-2	2	22.0	M	43
7-3	2	22.0	T	50
8-1	2	45.0	C	27
8-2	2	45.0	M	27
8-3	2	45.0	T	34
9-1	23	2.0	C	51
9-2	23	2.0	M	51
9-3	23	2.0	T	61
10-1	23	25.0	C	25
10-2	23	25.0	M	25
10-3	23	25.0	T	104

Table 14. Continued

Sample Number	Total Activity of Sample (μCi)	Activity per Egg or Membrane (μCi)	Activity Concentration in Egg Contents ($\mu\text{Ci/ml}$)	Concentration Factor for Egg Contents
1-1	3.0×10^{-3}	6.0×10^{-5}	1.4×10^{-2}	0.70
1-2	5.8×10^{-3}	1.2×10^{-4}		
1-3	9.1×10^{-3}	1.2×10^{-4}		
2-1	4.6×10^{-3}	8.7×10^{-5}	2.1×10^{-2}	1.05
2-2	7.8×10^{-3}	1.4×10^{-4}		
2-3	3.1×10^{-2}	3.1×10^{-4}		
3-1	8.7×10^{-3}	1.8×10^{-4}	4.3×10^{-2}	2.15
3-2	1.6×10^{-2}	3.4×10^{-4}		
3-3	2.2×10^{-2}	4.4×10^{-4}		
4-1	8.1×10^{-3}	2.6×10^{-4}	6.1×10^{-2}	3.05
4-2	1.4×10^{-2}	4.4×10^{-4}		
4-3	7.6×10^{-3}	1.1×10^{-3}		
5-1	3.6×10^{-3}	6.9×10^{-5}	1.6×10^{-2}	0.80
5-2	7.1×10^{-3}	1.3×10^{-4}		
5-3	1.4×10^{-2}	1.8×10^{-4}		
6-1	5.3×10^{-3}	1.1×10^{-4}	2.6×10^{-2}	1.30
6-2	1.4×10^{-3}	2.9×10^{-4}		
6-3	1.6×10^{-2}	2.6×10^{-4}		
7-1	8.8×10^{-3}	1.1×10^{-4}	2.6×10^{-2}	1.30
7-2	1.5×10^{-2}	3.5×10^{-4}		
7-3	2.3×10^{-2}	4.6×10^{-4}		
8-1	4.8×10^{-3}	1.8×10^{-4}	4.3×10^{-2}	2.15
8-2	1.6×10^{-2}	5.9×10^{-4}		
8-3	3.9×10^{-2}	1.1×10^{-3}		
9-1	5.6×10^{-3}	1.1×10^{-4}	2.6×10^{-2}	1.30
9-2	5.7×10^{-3}	1.1×10^{-4}		
9-3	1.1×10^{-2}	2.2×10^{-4}		
10-1	3.5×10^{-3}	1.4×10^{-4}	3.4×10^{-2}	1.70
10-2	6.8×10^{-3}	2.7×10^{-4}		
10-3	4.4×10^{-2}	4.3×10^{-4}		

Table 15. Analytical Data for ^{233}U
Quantitative Penetration Studies

Sample Number	Time After Fertilization That Eggs Were Placed in the Test Solution (hours)	Time Eggs Remained in the Test Solution (hours)	Type of Sample C=Contents M=Membrane T=Total Egg	Sample Size
1-1	0	2.0	C	52
1-2	0	2.0	M	52
1-3	0	2.0	T	100
2-1	0	9.0	C	52
2-2	0	9.0	M	52
2-3	0	9.0	T	103
3-1	0	25.0	C	50
3-2	0	25.0	M	50
3-3	0	25.0	T	50
4-1	0	49.0	C	40
4-2	0	49.0	M	40
4-3	0	49.0	T	50
5-1	2	2.0	C	63
5-2	2	2.0	M	63
5-3	2	2.0	T	100
6-1	2	8.0	C	50
6-2	2	8.0	M	50
6-3	2	8.0	T	101
7-1	2	25.0	C	53
7-2	2	25.0	M	53
7-3	2	25.0	T	101
8-1	2	48.0	C	31
8-2	2	48.0	M	31
8-3	2	48.0	T	50
9-1	25	3.0	C	50
9-2	25	3.0	M	50
9-3	25	3.0	T	82
10-1	25	18.0	C	27
10-2	25	18.0	M	27
10-3	25	18.0	T	53

Table 15. Continued

Sample Number	Total Activity of Sample (μCi)	Activity per Egg or Membrane (μCi)	Activity Concentration in Egg Contents ($\mu\text{Ci}/\text{ml}$)	Concentration Factor for Egg Contents
1-1	9.4×10^{-4}	1.8×10^{-5}	4.3×10^{-3}	0.36
1-2	1.1×10^{-3}	2.1×10^{-5}		
1-3	3.6×10^{-3}	3.6×10^{-5}		
2-1	2.1×10^{-3}	4.1×10^{-5}	9.7×10^{-3}	0.81
2-2	6.2×10^{-4}	1.2×10^{-5}		
2-3	3.0×10^{-3}	2.9×10^{-5}		
3-1	1.0×10^{-3}	2.1×10^{-5}	4.9×10^{-3}	0.41
3-2	6.5×10^{-4}	1.3×10^{-5}		
3-3	1.9×10^{-3}	3.8×10^{-5}		
4-1	2.3×10^{-4}	5.9×10^{-6}	1.4×10^{-3}	0.12
4-2	1.9×10^{-4}	4.9×10^{-6}		
4-3	5.9×10^{-4}	1.2×10^{-5}		
5-1	3.5×10^{-4}	5.6×10^{-6}	1.3×10^{-3}	0.11
5-2	2.0×10^{-4}	3.2×10^{-6}		
5-3	1.2×10^{-3}	1.2×10^{-5}		
6-1	2.5×10^{-3}	5.0×10^{-5}	1.2×10^{-2}	1.00
6-2	3.7×10^{-3}	7.3×10^{-6}		
6-3	3.0×10^{-3}	3.0×10^{-5}		
7-1	1.4×10^{-3}	2.7×10^{-5}	6.3×10^{-3}	0.53
7-2	1.6×10^{-3}	2.9×10^{-5}		
7-3	6.7×10^{-3}	6.6×10^{-5}		
8-1	1.5×10^{-4}	4.9×10^{-6}	1.2×10^{-3}	0.10
8-2	1.1×10^{-4}	3.7×10^{-6}		
8-3	1.7×10^{-3}	3.4×10^{-5}		
9-1	1.6×10^{-3}	3.1×10^{-5}	7.4×10^{-3}	0.62
9-2	1.1×10^{-3}	2.2×10^{-5}		
9-3	3.1×10^{-3}	3.8×10^{-5}		
10-1	1.3×10^{-4}	5.0×10^{-6}	1.2×10^{-3}	0.10
10-2	1.7×10^{-4}	6.3×10^{-6}		
10-3	3.5×10^{-3}	6.5×10^{-5}		

APPENDIX C
DATA FOR TOXICITY EXPERIMENTS

Table 16. Data for Toxicity Experiments (Carp/ ^{238}Pu)

Treatment ($\mu\text{Ci/ml}$)	Number Eggs Number Dishes	Mean Percent Hatch (%)	Number Abnormal	Standard Error of Mean Hatch	Significant Number of Abnormals at 95% Level ^a	Comments
7.5	563/3	84	See comments	1.5		Most were abnormal
control	628/3	96	1	0.8		
3.9	437/3	96	19	1.0	yes	
control	628/3	96	1	0.8		
1.6	377/3	98	5	1.9	yes	
control	380/3	98	0	0.8		
0.16	312/3	97	2	0.9		
control	380/3	98	0	0.8		

^aAs determined by Fisher's Exact Test.

Table 16. Data for Toxicity Experiments (Fathead Minnows/ ^{238}Pu)

Treatment ($\mu\text{Ci/ml}$)	Number Eggs Number Dishes	Mean Percent Hatch (%)	Number Abnormal	Standard Error of Mean Hatch	Significant Number of Abnormals at 95% Level	Comments
6.5	185/2	0	0			Eggs dead by 48 hrs.
control	251/2	89	0	2.0		
3.2	313/3	0	0			Hatched prematurely
control	315/3	74	0	2.5		
1.3	313/3	2	0	0.8		Hatched prematurely
control	272/3	90	0	1.8		
0.85	369/3	77	See comments	2.2	yes	Most were abnormal
control	343/3	79	1	2.2		
0.26	311/3	86	See comments	2.0	yes	Most were abnormal
control	340/3	88	0	1.8		
0.076	454/3	81	15	1.8	yes	
control	408/2	77	0	2.1		
0.02	326/3	92	0	1.5		
control	365/3	87	0	1.8		
0.006	484/3	90	0	1.3		
control	169/1	88	0	2.5		

Table 16. Data for Toxicity Experiments (Carp/ ^{232}U)

Treatment ($\mu\text{Ci/ml}$)	Number Eggs Number Dishes	Mean Percent Hatch (%)	Number Abnormal	Standard Error of Mean Hatch	Significant Number of Abnormals at 95% Level	Comments
12.5	504/3	0	0			Eggs dead by 48 hrs.
control	628/3	96	0	0.8		
5.0	592/3	0	0			Developed to hatching
control	628/3	96	0	0.8		
1.2	421/3	97	12	0.8	yes	
control	628/3	96	0	0.8		
0.8	667/3	95	0	0.9		
control	628/3	96	0	0.8		

Table 16. Data for Toxicity Experiments (Fathead Minnows/ ^{232}U)

Treatment ($\mu\text{Ci/ml}$)	Number Eggs Number Dishes	Mean Percent Hatch (%)	Number Abnormal	Standard Error of Mean Hatch	Significant Number of Abnormals at 95% Level	Comments
18.6	533/3	0	0			Eggs dead by 48 hrs.
control	318/3	92	0	1.5		
8.9	239/3	0	0			70% dead by 24 hrs.
control	257/3	81	0	2.4		
4.4	315/3	0	0			
control	257/3	90	1	1.9		
2.9	425/3	0	0			Eggs dead by 96 hrs.
control	321/3	72	0	2.5		
1.1	320/3	0	0			
control	307/2	82	1	2.2		
0.5	154/2	0	0			
control	169/2	80	0	3.1		
0.2	415/3	76	See comments	2.1		Most were abnormal
control	260/3	94	0	1.5		
0.05	238/3	83	5	2.4	yes	
control	266/3	87	0	2.0		
0.01	269/3	88	0	2.0		
control	252/3	79	0	2.6		

Table 16. Data for Toxicity Experiments (Carp/ ^{244}Pu)

Treatment (ppm)	Number Eggs Number Dishes	Mean Percent Hatch (%)	Number Abnormal	Standard Error of Mean Hatch	Comments
19.0	468/3	0	0		Died before eyed stage
control	628/3	96	0	0.8	
9.0	218/2	84	0	2.5	Hatch delayed 6 hrs.
control	380/3	98	0	0.8	
0.7	473/3	96	1	0.9	
control	380/3	98	0	0.8	

Table 16. Data for Toxicity Experiments (Fathead Minnows/ ^{244}Pu)

Treatment (ppm)	Number Eggs Number Dishes	Mean Percent Hatch (%)	Number Abnormal	Standard Error of Mean Hatch	Comments
20.0	239/1	0	0		Eggs dead by 4th day
control	141/1	84	0	3.4	
10.0	79/1	87	0	4.0	
control	73/1	99	0	1.2	
5.7	119/1	97	0	1.5	
control	84/1	98	0	1.5	
1.1	104/1	79	0	4.5	
control	166/1	77	0	3.7	

Table 16. Data for Toxicity Experiments (Carp/ ^{233}U , ^{235}U , ^{238}U)

Treatment (ppm - isotope)	Number Eggs Number Dishes	Mean Percent Hatch (%)	Number Abnormal	Standard Error of Mean Hatch	Comments
6- ^{233}U	522/3	95	3	0.9	
control	410/3	97	0	0.9	No citrate added
10- ^{238}U	528/3	82	6	1.7	
control	467/3	94	3	1.1	No citrate added
20- ^{238}U	531/3	91	2	1.3	
control	467/3	94	3	1.1	No citrate added
40- $^{235}, ^{238}\text{U}$	677/3	94	0	0.9	
control	628/3	96	0	0.8	No citrate added
60- $^{235}, ^{238}\text{U}$	667/3	95	0	0.8	
control	628/3	96	0	0.8	No citrate added
70- ^{233}U	363/3	76	20	2.1	
control	410/3	97	0	0.9	No citrate added

Table 16. Data for Toxicity Experiments (Carp/ $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$)

Treatment (% $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$)	Number Eggs Number Dishes	Mean Percent Hatch (%)	Number Abnormal	Standard Error of Mean Hatch	Comments
1×10^{-1}	373/3	0	0		Eggs dead by 24 hrs.
control	467/3	94	3	1.1	
5×10^{-2}	555/3	18	1	1.6	Delayed hatching 24 hrs.
control	467/3	94	3	1.1	
1×10^{-2}	485/3	90	0	1.4	Delayed hatching
control	467/3	94	3	1.1	
5×10^{-3}	457/3	97	0	1.0	
control	410/3	92	0	1.3	
1×10^{-3}	528/3	95	0	1.0	
control	410/3	92	0	1.3	
5×10^{-4}	520/3	94	0	1.0	
control	410/3	92	0	1.3	
1×10^{-4}	599/3	91	0	1.2	
control	410/3	92	0	1.3	

Table 16. Data for Toxicity Experiments (Carp/KF)

Treatment (ppm F ⁻)	Number Eggs Number Dishes	Mean Percent Hatch (%)	Number Abnormal	Standard Error of Mean Hatch	Comments
1900	425/3	0	0		
control	344/2	93	0	1.3	
950	492/3	0	0		
control	344/2	93	0	1.3	
190	431/3	55	0	2.4	Larvae lived 12 hrs.
control	344/2	93	0	1.3	
95	690/3	95	0	0.8	
control	344/2	93	0	1.3	
19	612/3	96	0	0.8	
control	344/2	93	0	1.3	
9.5	493/3	97	0	0.8	
control	344/2	96	0	1.3	

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